INHERITANCE IN CROSSES BETWEEN *NICOTIANA LANGSDORFFII* AND *NICOTIANA ALATA*

E. M. EAST

Harvard University, Bussey Institution, Forest Hills, Massachusetts

[Received March 14, 1916]

TABLE OF CONTENTS

	PAGE
INTRODUCTION	311
Early work	313
Inheritance of pollen color and of flower color	317
Fertility of the hybrids	319
Height	319
Rapidity of growth	320
Leaves	320
Corolla length	322
The reciprocal cross	329
CONCLUSION	332
LITERATURE CITED	333

INTRODUCTION

In a rather intensive genetic study of the genus Nicotiana including some sixty inter-specific crosses, the writer has found very few fertile crosses between species whose status would not be questioned by taxonomists. Of these, the one showing the most perfect fertility is to be described in this paper.

Nicotiana Langsdorffii Weinm. and *Nicotiana alata* Lk. and O. are so different from each other in their characters that they were placed by GEORGE DON in the different sections of the genus, that he called Rustica and Petuniodes, and have been kept there by COMES, the most recent monographer of the Nicotianas. The writer agrees with the suggestion of LOCK (1909) that *N. Langsdorffii* should be removed from the Rustica section to the Petuniodes section on the basis of its genetic behavior when crossed with *N. alata,* but the very fact that taxonomists without access to genetic data have seen fit thus to separate them is an indication of a specific distinction not to be questioned except by those who would fuse all types giving fertile hybrids.

Nicotiana Langsdorffii was described by WEINMANN (ROEM. &

SCHULT. Syst. iv. p. 323) from Brazil. It probably has a wide distribution in South America as it has been found in Chile (COMES 1899). The immediate sources of my plants were SETCHELL (1912, his $\frac{3}{0}\frac{3}{2}$) and A. SPLENDORE, Scafati, Italy. I do not know where SETCHELL obtained

FIGURE 1

FIGURE 2

FIGURE 1. A young flowering plant of *Nicotiana alata* Link and Otto, var. *grandiflora* Comes.

FIGURE 2. A young flowering plant of *Nicotiana Langsdorffii* Weinm.

his plants, and the two strains may be from the same stock. At any rate they are practically identical, both corresponding with the plate in the BOTANICAL MAGAZINE (1825 pl. 2555).

The plants are from 120—145 cm in height, vigorous, profusely branched, the branches erect. The basal leaves are 20—30 cm long,

obtuse, ovate, sessile, narrowed and decurrent at the base. Upper leaves are lanceolate, and all are extremely rugose above. Inflorescence racemo-paniculate. Flowers are about 20 mm long, very uniform in size; corollas funnel-shaped, a gibbous ring above, the limb concave, spreading, and very slightly notched; greenish yellow, pendulous. The pollen is blue.

Nicotiana alata as described by LINK and OTTO (see Ic. Pl. Rar. I, 63, t. 32. DC. Prodr. XIII. I. p. 567. GARTENFLORA tab. 1010. COMES 1899, p. 35) from Brazil (found in Uruguay and Paraguay according to COMES), I have never seen. The type used in these experiments is the common *N. affinis* Moore (GARDN. CHRON., 1881, p. 141) referred by COMES to the variety *grandiflora*. The variety seems to have no points by which it can be distinguished from the species. It is described as having larger flowers with more perfume, more zygomorphism and less gibbosity than the species, but these are very indefinite and inconstant qualities.

The strain with which our crosses were made has plants 110-130 cm high, appearing shorter because of the loosely spreading habit. Basal leaves are acute, ovate, quickly narrowed to a slightly decurrent base, slightly rugose; upper leaves lanceolate to linear. Inflorescence is a raceme. Flowers are 75—95 mm long, tube gradually enlarging toward the limb and slightly gibbous at the top, light greenish yellow faintly lined with purple; limb broadly expanded into obtuse, ovate lobes the lower two being distinctly smaller than the other three and giving the flower a decidedly zygomorphic form. The corolla limb is pure white on the inside and cream with sometimes a tinge of purple on the outside. One anther is usually somewhat shorter than the others. The pollen is white or yellowish. Some plants are self-fertile, others are completely self-sterile.

EARLY WORK

These two species were crossed and studied by at least three of the earlier hybridizers, NAUDIN, GODRON and FOCKE. Concerning their results, I quote FOCKE (1881):

"*N. alata* Lk. × *Langsdorffii* Weinm. GÄRTNER found no foreign species with which he was able to fertilize *N. Langsdorffii*. Reciprocal crosses between *N. alata* and *N. Langsdorffii* are not difficult, however; NAUDIN obtained especially good, well filled capsules by fertilizing *N. Langsdorffii* with pollen from *N. alata*, and although only one pollination of *N. alata* with *N. Langsdorffii* pollen was successful, in this case also a large capsule full of seeds matured. I found no difficulty with either cross. Of *N.*

Langsdorffii ♀ × *N. alata* ♂ (*N. Persico-Langsdorffii* Naud. l. c. p. 74) NAUDIN produced 118, and of *N. alata* ♀ × *N. Langsdorffii* ♂ (*N. Langsdorffii-Persica* Naud.) 53 examples; all of which were exactly like one another. They were 130—160 cm high (*N. Langsd.* ca. 100; *N. alata* 60 cm) and because of their spreading branches more nearly resembled *N. Langsdorffii*. The blossoms were medium large, greenish white, with the limbs distinctly rounded. Pollen bluish gray. Fruitfulness perfect. I have made the same crosses with like results. Pollen was plentiful and the grains well-formed. The capsules contained in the neighborhood of 500 seeds.

"NAUDIN's hybrids were to be distinguished from *N. commutata* by their higher stature, their larger and more greenish flowers, and their darker leaves.

"*Later generations.* Through continued self-fertilization, NAUDIN's hybrid plants gradually returned toward the condition of the parent species, although this was never fully reached. GODRON received from ALEX. BRAUN of Berlin, seed of *N. alata-Langsdorffii* (as well as of *N. Langsdorffii* ♀ × *N. alata* ♂) and raised many forms from it; among others were varieties with yellow, with cream, and with pure white flowers. The leaves were variable, the decurrence at the stem being sometimes very pronounced, sometimes just traceable and sometimes lacking.

"Two varieties in which crossing had been prevented by gauze produced fruits whose seeds reproduced the mother form exactly."

From this extract, it is clear that FOCKE was familiar with the facts that in the cross under consideration—as well as in other crosses—the F_1 generation is more vigorous than either of the parent species, that the population is uniform and the individual plants fertile, and finally that the F_2 and following generations are variable and may produce plants having a striking resemblance to the original parents. In this he was merely copying NAUDIN. Both NAUDIN and GODRON perceived the essential facts of inheritance in hybrids much more clearly than other contemporary hybridizers, and we may be assured that had NAUDIN had an opportunity of reading MENDEL's paper, as did NÄGELI, he would have appreciated its significance. He came very close to an enunciation of what we now know as the Mendelian laws, but either he lacked the ability for mathematical analysis that characterized MENDEL because of the latter's training in physics, or was prevented from making such an analysis by the greater complexity of the hybrids he studied.

NAUDIN (1865) says of his cross, *Nicotiana Persico-Langsdorffii:*

"The two plants here united, although very different at first sight, have distinct analogies in their habit of growth, the form of their leaves, their general aspect, and up to a certain point, in their long, tubular, pendent flowers. One feels these analogies more strongly if he remembers that there exists a form exactly intermediate between the two (*N. commutata*, Fisch.), of which I shall speak later. As well as I can judge by the descriptions, *N. Persica*, of which there is a pretty good figure in the BOTANICAL REGISTER, pl. 1592, appears to be identical with the *N. alata* of DUNAL;

not being sure, however, I have preserved the name that it carried at the Museum."

NAUDIN wished to see whether *N. commutata* was a natural hybrid and if he could reproduce it by this cross. He obtained 118 plants from his cross, "all of the most uniform appearance" and from 130-160 cm high. He concludes that *N. commutata* is not the hybrid *N. Persico-Langsdorffii,* "at least of the first generation." Since he made this conclusion solely on account of the greater height and vigor of his artificial hybrids, it must be that he suspected that he might have duplicated *N. commutata* when by inbreeding his plants had lost their hybrid vigor. I have duplicated plants of *N. commutata* grown from seed received from Dr. SPLENDORE of Scafati, Italy, several times in my own crosses and have obtained F_3 families that bred as true to the form (intermediate between *N. alata grand.* and *N. Langsdorffii*) of the so-called *N. commutata* as did the species (?) itself.

NAUDIN found that the reciprocal cross was so nearly like the other that "without the labels the two lots would have been taken the one for the other." Unfortunately, however, although these crosses were perfectly fertile, NAUDIN did not self them and continue his observations. On the other hand, he did obtain some information regarding later generations by a consideration of the volunteer seedlings that appeared during the next few years on the plat that had borne the original cross. He says:

"Without having given these hybrids of the second and the third generations the attention they merited, I have noticed that their forms became more and more divergent, some approaching *N. Persica* and others distinctly tending toward *N. Langsdorffii.*"

Some of these plants he potted, and obtaining seed from one that resembled *N. Langsdorffii* he grew a population that bred true to a type that could scarcely be distinguished from *N. Langsdorffii.* These experiments were continued, and from seed of this generation, he raised in 1863, fifty plants nearly all of which "had returned to the type well known as *N. Langsdorffii.*"

Thus it is seen that NAUDIN observed nearly all the essential facts of Mendelian heredity in this one cross,—a uniform F_1 generation, a segregating F_2 generation, and a later generation which showed that certain of the extreme segregates bred true. But the observations on this particular cross are not so important as the general conclusions to which NAUDIN was led by his broad experience as a hybridizer. Under the heading "Physionomie des hybrides," he says:

"In order to have a correct idea concerning the phenomena presented by hybrids, it is essential to distinguish between the first generation and those that follow.

"I have always found, in the hybrids I have made myself or of whose origin I was certain, a great uniformity of aspect between individuals of the first generation and originating from the same cross no matter what their number. This fact we have seen exemplified in *Petunia violaceo-nyctaginiflora, Datura Tatulo-Stramonium* and *D. Stramonio-Tatula, D. Meteloido-Metel, D. Stramonio-lævis,* etc., *Nicotiana Texano-rustica* and *rustico-Texana, N. Persico-Langsdorffii,* etc.; having already emphasized these resemblances it is useless for me to dwell upon them."

"In fact, one may say that hybrids of the first generation resemble each other as much or nearly as much as the individuals that come from a single legitimate species."

(It is well to note that the hybrids with which NAUDIN supports his thesis here are all between solanaceous species that are generally self-pollinated naturally, and may be presumed to approach homozygosis.)

"Beginning with the second generation, the aspect of hybrids is changed in a remarkable manner. Ordinarily, the perfect uniformity of the first generation is succeeded by a regular medley of forms, some approaching the specific type of the father, others that of the mother, a few returning suddenly and entirely to the one or the other form. At other times, this progress toward the original types is by degrees and slowly, and sometimes one sees a whole collection of hybrids incline toward the same side. The important fact, however, is, that it is the second generation, in the great majority of cases (and perhaps in all), that starts this dissolution of the hybrid forms, a phenomenon recognized by many investigators, doubted by others, but which appears to me to-day to be established beyond argument. We shall explain the cause in the following paragraph."

"All of the hybrids of which I have studied the second generation with some care, have shown these changes in appearance and have manifested this tendency to return to the forms of the original species, and this when conditions have been such that the pollen of the species themselves could not have been the cause. We have seen striking examples of it in *Primula officinali-grandiflora,* in all of the hybrids of *Datura Stramonium,* in *D. Meteloido-Metel,* in the reciprocal hybrids of *Nicotiana angustifolia* and *macrophylla, N. Persica* and *Langsdorffii, Petunia violacea* and *nyctaginiflora,* in *Luffa acutangulo-cylindrica,* and further in *Linaria purpureo-vulgaris.* In the second generation of several of these hybrids there has been a complete return to one or the other or to both of the parent species together with individuals approaching each species in varying degrees; in other cases also we have seen intermediate forms continued at the same time that other specimens from the same family have effected the return of which I speak. Moreover, we have observed cases (*Linaria purpureo-vulgaris* of the third or fourth generation) of actual retrogression toward the hybrid form, sometimes a plant that had apparently returned entirely to one of the two species, has even given rise to individuals that very nearly resembled the other species. All of these facts are explained naturally by the *segregation* (disjunction) *of the two specific essences* in the pollen and the ovules of the hybrid."

Space may not be claimed to show just how NAUDIN's views differed from those of MENDEL, except the bare statement that he did not grasp the idea of a unit-character inheritance. Our quotations are already somewhat lengthy. They may well be pardoned, however, since they are taken from a paper not readily available to most geneticists, and have a considerable theoretical and historical interest. But it was not for this alone that I have used them, nor because they contain observations upon the particular cross that is the subject of this paper. And in passing let me say that there is scarcely a doubt but that NAUDIN's *Nicotiana Persica* and *N. Langsdorffii* are the same as our own *N. alata grandiflora* and *N. Langsdorffii*. The particular reason for the citations is this: While it is to be hoped that with the fruitful hypotheses of modern biology as guides, contemporary genetic research is to be more productive than that of the early nineteenth century hybridizers, it must not be forgotten that very often we are merely repeating more carefully, more quantitatively and with a better idea of relative values, the experiments of these pioneers. The observations of such men as NAUDIN have been confirmed and as far as they go are usually correct. For this reason I think that we may accept their facts until the same experiments have been repeated more carefully and have given us more precise data. This being true, there is no question but that these numerous observations on hybrids between species belonging to so many different groups, showing as they do all the essential phenomena of Mendelian inheritance, go far toward proving Mendelian heredity in quantitative characters.

The only recent work upon *N. Langsdorffii-alata* hybrids is that of LOCK. LOCK made a number of crosses between species of Nicotiana in the years 1906-8, but published only one paper (1909) on the subject. He crossed *N. alata* and *N. Langsdorffii* reciprocally, made several back-crosses, and studied the selfed progeny of the F_1 generation. He noted the uniformity of the F_1 generation and the variability of the F_2 generation and reported a few measurements of the flowers. He established the dominance of blue pollen over yellow and of yellow corollas over white. He also believed that the facts indicated the dominance of gibbous over funnel-shaped corollas.

INHERITANCE OF POLLEN COLOR AND OF FLOWER COLOR

In a cross such as this between two distinct species it is important to know whether any distinct qualitative difference shows a Mendelian behavior. I found only two such differences, pollen color and flower color,

and have corroborated the results of Lock in regard to them. I was not able to corroborate his conclusions in regard to corolla shape, as F_2 plants all showed some development of the gibbous condition. And it seems to me that this was to be expected for it is characteristic of both species.

N. alata has yellow pollen and *N. Langsdorffii* blue pollen. No matter which way the cross is made the pollen is blue. The pollen of the heterozygotes is often lighter than that of the pure *N. Langsdorffii,* but not invariably so. Microscopical examination showed no distinctly yellow grains on the F_1 plants so that, like pollen shape in Lathyrus, the color is a sporophytic character. This is less astonishing than the phenomenon in the sweet pea, for it is well known that pollen color is a tapetal deposit. One F_2 consisted of 342 plants with blue pollen and 100 plants with yellow pollen. Counts of smaller segregating populations corroborated these results, though there was an excess of blues in all but one case, an F_3 family consisting of 39 blue and 22 yellow. Just what this excess of blue-pollened plants means, I am unable to say. It may be only a technical difficulty, as the anthers of both species are blue. At any rate, there seems to be no possibility of other factors being concerned directly. Yellow-pollened plants have never given blues.

The flower of *N. Langsdorffii* is greenish yellow both outside and inside the corolla. *N. alata,* on the other hand, though slightly greenish yellow with sometimes a faint tinge of purple on the outside of the corolla, is pure white on the inside of the limb. Apparently the cells just beneath the epidermis on the inside of the limb of these flowers contain no colored chromatophores and very few plastids of any sort. The flowers of the F_1 hybrids (made either way) are cream-colored, but appear to be variable because the old flowers are so light as to be sometimes mistaken for whites. Further the smaller-flowered plants appear to be a darker yellow owing to a concentration of chromoplasts which show through the upper two layers of cells. There is no question but that the inheritance of these differences is Mendelian, but it is not certain that only one factor is involved. Three F_2 populations gave ratios of 196 yellows to 61 whites, 50 yellows to 15 whites, and 57 yellows to 15 whites respectively. A heterozygous F_3 family also gave a ratio of 112 yellows to 29 whites, but one of our F_2 families produced 70 yellows to 6 whites. This constant excess of yellows leads one to suspect complications, but it can be said that no white ever produced yellows after self-pollination, though a number of such families were grown.

It is possible that there is a correlation between small flowers and yellow color though this could not be established.

FERTILITY OF THE HYBRIDS

These slightly distorted ratios give some cause for the surmise that differential fertility exists among the gametes and the zygotes. That all the possible gametic constitutions mature at spermatogenesis cannot be asserted without a cytological study of the early stages. The capsules on the F_1 plants were well filled, however, and the germination of the seeds was between 90 percent and 100 percent.[1] This seems a fair proof that the ovules were all functional and that there was no selective elimination of zygotes.

On the other hand, all of the pollen produced by the F_1 plants and of the plants of later generations was not well formed. An examination of the pollen of 20 F_1 plants after having simply shaken it out on slides showed both when dry and in glycerin or in sugar solutions that apparently functional pollen grains existed in percentages varying from 70 to 96. Of course one cannot say that all of these seemingly well-formed pollen grains are functional, as DORSEY (1915) has shown that in certain Vitis species they sometimes contain no generative nuclei; but since in nearly all the plants there are around 85-90 percent perfectly formed pollen grains one may be fairly certain that if much selective elimination of gametes occurs it occurs before the pollen grains are formed, for the parent species themselves show only from 80-90 percent of well formed grains.

HEIGHT

Nicotiana Langsdorffii (328) and *N. alata* (321) are nearly the same height,—about 132 cm and 120 cm respectively,—but they are very difficult to measure owing to their becoming so profusely branched during

[1] GOODSPEED (1913) has criticized a table published by EAST and HAYES (1912, p. 28) entitled "Condition of hybrids in crosses between species of Nicotiana" because a number of Nicotiana hybrids were tabled as showing 100 percent germination. This table was published to indicate the general type of certain hybrids with regard to vigor, and I think served its purpose. It was distinctly stated, however, (p. 29) that "the voluminous data that have been collected on these hybrids have been condensed and approximated so that they include only facts germane to the matter in hand." It would seem that it might have been clear to GOODSPEED from this statement that these germinations were only classes. Possibly it would have been better to have said germination "high, medium, low and failing," but it does not seem to me that the readers were led far astray. As a matter of fact the germinations tabled as 100 percent, included all hybrids that tested over 90 percent.

the latter part of the season. The F_1 generation was as uniform as either parent and showed distinct evidence of hybrid vigor. The plants varied from 140 cm to 160 cm. In the second hybrid generation there was no evidence of segregation into distinct types, either as regards height or general habits of growth but plants varied from below the height of No. 321 (extremes about 100 cm) to that of the F_1 generation.

RAPIDITY OF GROWTH

Though both of these species continue flowering until frost, *N. Langsdorffii* commences flowering earlier than *N. alata,* when planted at the same time, and owing to the multitude of ripe capsules formed, takes on a more mature appearance in September. The variation in time of flowering within each species is very slight. In seasons with normal rainfall, sunlight and heat, plants of *N. Langsdorffii* planted in the greenhouse at the same time and set in the field on the same day, come into blossom within three days of each other. Plants of *N. alata* treated in the same manner, show greater variation, sometimes a week elapsing between the time that extremes begin blossoming. The F_1 plants are as uniform in this respect as *N. Langsdorffii* and are slightly earlier. The F_2 plants, on the other hand, are more variable than those of *N. alata,* and this variability is not wholly an effect of environment as is beautifully demonstrated by the F_3 cultures. Ten progeny rows from different F_2 plants showed a difference of 25 days in the time the plants began to flower. Four of the families were variable like the F_2 population, but the remainder were very uniform within the family. On the fifteenth of July two families were in full blossom without an exception, one family had just begun to bloom, one family had the central stalks well advanced and two families were in the rosette stage.

LEAVES

In general the shape of the leaves of both of these species is the same. The basal leaves of *N. alata,* however, are acute, with redundant, folded margins, while those of *N. Langsdorffii* are obtuse and not redundant at the margins. *N. Langsdorffii* is much more rugose than *N. alata.* Both species are decurrent. The tips of the leaves of the F_1 plants are intermediate, but in other qualities the leaves are like those of *N. alata.* The F_2 plants run the whole gamut of these variations. There are plants, the rugosity of whose leaves is like *N. Langsdorffii,* that are like *N. alata* in other respects (except that the flowers are smaller).

Table 1

Frequency distribution of corolla length in the families of cross 328 × 321.

Pedigree Number	Size in parent	Class Centers in millimeters																								No.
		19	22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	
328, (1914)		10	41																									51
328, (1911+1914)		13	56																									69
321, (1911)																				3	8	10	11	8	5	3	1	49
(328×321) F_1							1	4	24	16	1																	46
(328×321)−1 F_2	41		1	1	5	35	52	58	45	35	13	6	2	1	0	1	1											256
(328×321) total F_2			2	3	13	65	110	132	107	82	33	15	6	7	0	4	2											581
(328×321) 1−1 F_3	23		40	10																								51
(328×321) 1−2 F_3	37					1	24	25																				50
(328×321) 1−3 F_3	40						4	11	26	6	1																	48
(328×321) 1−4 F_3	63								1	3	14	10	17	11	7	5	3											71
(328×321) 1−41 F_3	60							1	1	5	12	17	9	11	7	2	1	0	0	0	1							67
(328×321) 1−5 F_3	54							2	0	7	5	2	1	3	1	2	1	1										25
(328×321) 1−6 F_3	60							2	2	9	27	29	29	17	28	15	3	3	1	3								168
(328×321) 1−7 F_3	21	11	39																									50

Those plants that have returned to the *N. Langsdorffii* habit and size of flower, however, always have rugose leaves though they may be acute.

In all of these respects the reciprocal crosses are so nearly alike that it is impossible to distinguish between them.

COROLLA LENGTH

As I have explained in other papers (EAST 1916 a, b), corolla length is an excellent character upon which to make genetic studies because of the very slight effect produced by environmental differences. Corolla measurements of single flowers when taken with due precautions as to uniformity of age of plant, age of flower, position of flower, etc., well represent the phenotypes of the plants concerned.

Table 1 gives the frequency distributions of such measurements upon populations of the pure species, the cross when *N. Langsdorffii* was used as the female, a single F_2 population, the total of several F_2 populations and eight F_3 families. Only three of these distributions contain as many individual measurements as I should like, and one—(328 × 321)1-5—must be discarded entirely on account of the small number of plants.

The statistical constants for these distributions are shown in table 2.

TABLE 2

Statistical constants for the frequency distributions of corolla length shown in table 1.

Pedigree Number	Size in parent	Mean	S. D.	C. V.	No.
328, (1914)		21.41 ± .11	1.19 ± .08	5.56 ± .37	51
328, (1911 + 1914)		21.43 ± .10	1.17 ± .07	5.46 ± .31	69
321, (1911)		81.76 ± .49	5.08 ± .35	6.21 ± .42	49
(328 × 321) F_1		40.78 ± .22	2.20 ± .15	5.39 ± .38	46
(328 × 321) — 1 F_2	41	37.77 ± .24	5.63 ± .17	14.91 ± .45	256
(328 × 321) total F_2		38.30 ± .17	5.99 ± .12	15.64 ± .32	581
(328 × 321) 1 — 1 F_3	23	22.65 ± .12	1.24 ± .08	5.47 ± .37	51
(328 × 321) 1 — 2 F_3	37	35.44 ± .15	1.62 ± .11	4.57 ± .31	50
(328 × 321) 1 — 3 F_3	40	39.31 ± .25	2.54 ± .17	6.46 ± .44	48
(328 × 321) 1 — 4 F_3	63	52.04 ± .44	5.52 ± .31	10.61 ± .61	71
(328 × 321) 1 — 41 F_3	60	51.02 ± .51	6.16 ± .36	12.07 ± .71	67
(328 × 321) 1 — 5 F_3	54	49.24 ± 1.09	8.05 ± .77	16.35 ± 1.60	25
(328 × 321) 1 — 6 F_3	60	52.79 ± .35	6.79 ± .25	12.86 ± .48	168
(328 × 321) 1 — 7 F_3	21	21.34 ± .12	1.24 ± .08	5.81 ± .39	50

As may be seen, *N. Langsdorffii* (No. 328) has a very low variability. This is to be expected, for *N. Langsdorffii* is practically always self-fertil-

ized naturally. *N. alata* is often self-fertilized, but evidence of considerable cross-fertilization has been found by observing the actions of Sphingidae in the evening, by isolating plants, and by self-sterility studies. Though the coefficient of variability (6.21 ± .42 percent) is almost as

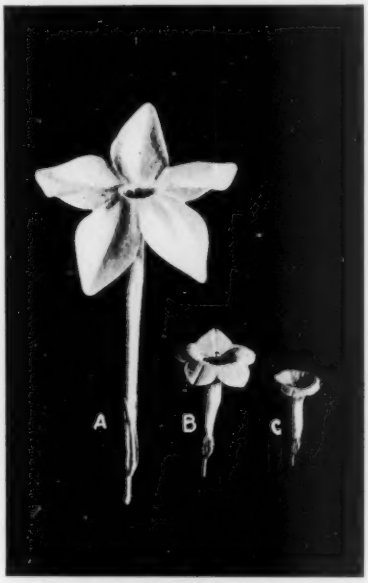


FIGURE 3

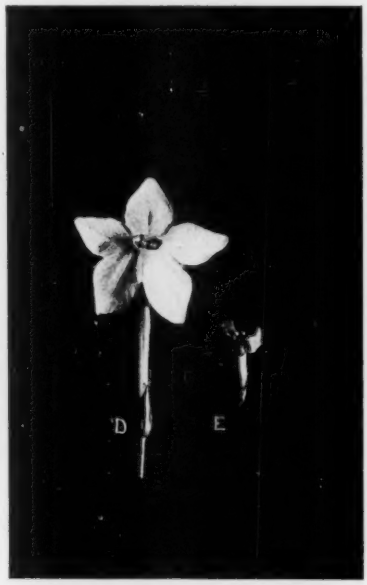


FIGURE 4

FIGURE 3. A, *N. alata grandiflora;* B, F_1 of *N. Langsdorffii* × *N. alata grandiflora;* C, *N. Langsdorffii* (1911) × ½.

FIGURE 4. D and E, extremes of the F_2 generation (1912) × ½.

low as that of No. 328, therefore, it is probable that No. 321 is not so nearly homozygous. Furthermore, the number of individuals measured is small. On the other hand, since a single plant of No. 321 was used in the cross, it is possible that the true variability of this "blood" introduced, is somewhat smaller than that represented by the frequency distribution.

Curiously enough the mean of the F_1 population is smaller than the average of the two parents. Thus there apparently is no effect of heterosis on the flowers. The square root of the F_1 mean is more nearly

that of the average of the square roots of the means of the two parents, but I do not feel justified in attaching any significance to the fact.

The coefficient of variation of the F_2 generation is nearly three times that of the F_1 generation. Though extremes like each parent were not produced, it is hardly possible to see any other cause for this great difference in variability than segregation and recombination of Mendelian factors. From the theory of probability one might expect to recover

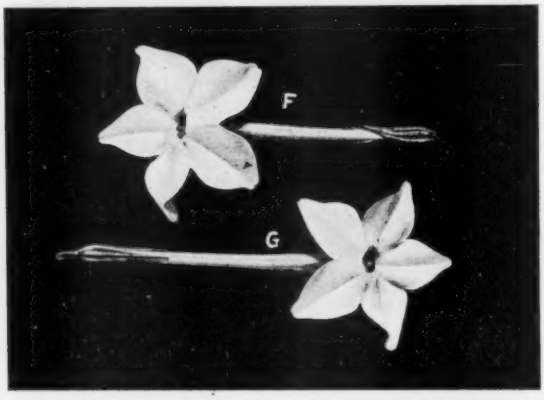


FIGURE 5. F, extreme of the F_2 generation, and G, pure *N. alata grandiflora* (1913) × ½.

both parents with a comparatively small number of F_2 plants, but the variability of F_1 is so small that even the plants obtained in F_2 could not be expected in the F_1 if the whole of New England were planted with them.

In the F_3 generation there was regression toward the mean of the F_2 population in six out of seven cases (excluding No. 1-5 on account of the small number of plants), *but the greatest extremes gave the least regression*. The coefficients of variability were lower than that of F_2 in every family, and three of them bred as true as the parental species. F_3 family No. 1-7 reproduced *N. Langsdorffii* exactly.

The Mendelian theory calls for the production of the same type of F_2 population no matter what F_1 parent is selected, when the original individuals entering the cross are homozygous. Critics of the use of the Mendelian terminology in crosses involving size characters have maintained, however, that small F_1 individuals will give F_2 populations

FIGURE 6

FIGURE 7

FIGURE 6. Individual plant produced by an F_2 segregate that was like *N. Langsdorffii* in every detail (E of figure 4).

FIGURE 7. Progeny row showing uniformity of F_3 family to which the plant shown in figure 6 belonged.

with lower means than will large individuals. As there are many indications that the plants entering into this cross were very nearly true homozygotes I have endeavored to test this proposition. Of course, as might be expected by pro-Mendelians, in such a cross the variability of the F_1 population is so low that the extremes selected differed by only 8 mm. Nevertheless five F_2 frequency distributions from different F_1 parents are presented in table 3. The statistical constants shown in table 4 emphasize the fact that the means and the standard deviations

Table 3

Frequency distributions of corolla length in five F_2 families of cross 321 × 328 — 1.

Pedigree Number	Size in parent	Class centers in millimeters															No.
		22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	
(328 × 321) — 1	41	1	1	5	35	52	58	45	35	13	6	2	1	0	1	1	256
(328 × 321) — A	44	1	1	3	9	27	25	24	11	2	3	1	1	0	1		109
(328 × 321) — 2	40			1	6	8	18	11	11	7	1	2	0	0	2		67
(328 × 321) — 3	36		1	2	7	13	18	16	12	6	1	0	1				77
(328 × 321) — 4	39			2	8	10	13	11	13	5	4	1	4	0	0	1	72

TABLE 4

Statistical constants of the corolla length in the five F_2 families reported in table 3

Number	Size in parent	Mean	S. D.	C. V.	No.
(328 × 321) — 1	41	37.77 ± .24	5.63 ± .17	14.91 ± .45	256
(328 × 321) — A	44	37.55 ± .36	5.65 ± .26	15.05 ± .70	109
(328 × 321) — 2	40	39.73 ± .52	6.32 ± .37	15.91 ± .95	67
(328 × 321) — 3	36	38.21 ± .40	5.21 ± .28	13.63 ± .75	77
(328 × 321) — 4	39	40.08 ± .56	7.11 ± .40	17.24 ± 1.00	72

very nearly overlap. In other words the curves are very nearly identical, and it can be shown mathematically that the probability is very high that they are all samples of the same population. The similarity of the curves is shown graphically in figure 9. The points of the theoretical curves of these five F_2 populations were calculated and are shown in

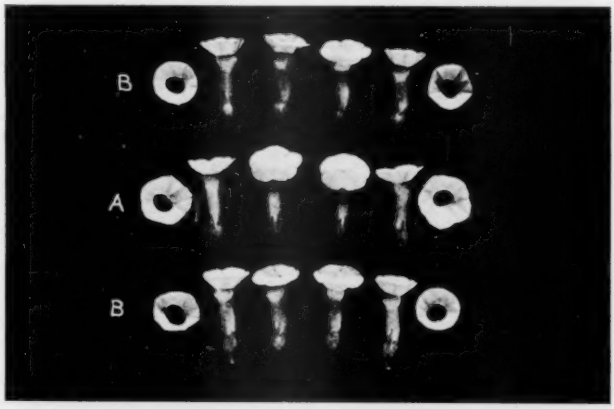


FIGURE 8. A, a random sample of *N. Langsdorffii* flowers from six different plants; B, a random sample of flowers from twelve different plants from the progeny row shown in figure 7 (× ½).

comparison with the theoretical curve of the total distribution of all F_2 observations. The extreme classes are so nearly identical that curves could not be distinguished when drawn super-imposed, so that only the points are indicated. Where no points for a particular curve are given it is understood that they lie on the single curve of total observations which is drawn.

Certainly no one can well maintain that these curves show any decided difference when the probable errors are taken into consideration. There is no dissimilarity in variability like that shown by F_3 populations from different points on the F_2 curve. The mean of the population from the 36-mm parent is higher than that from the 44-mm parent. This fact is not to be taken as significant; it is merely a coincidence. It is very evident that the only just conclusion is that selection has no effect.

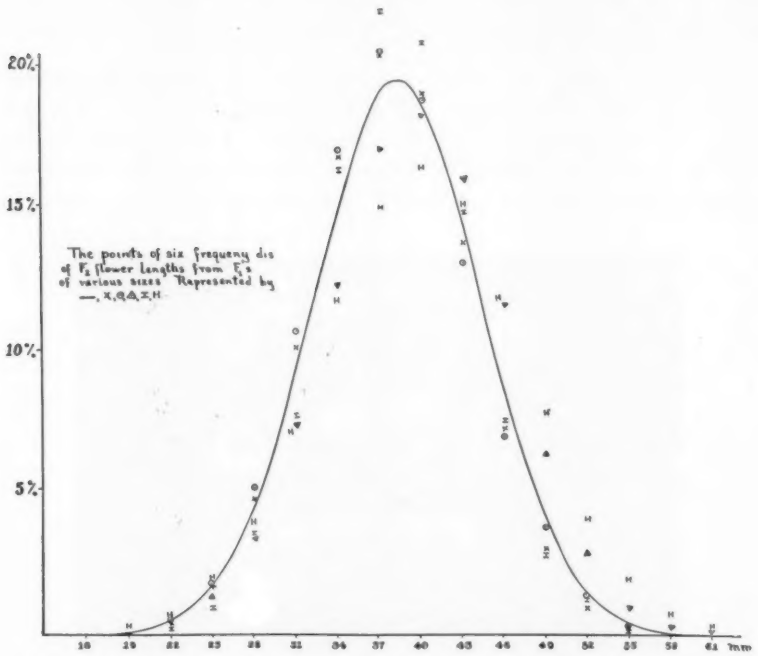


FIGURE 9. The points of the theoretical curves for flower length, calculated from the data obtained from growing F_2 populations from F_1 individuals of various sizes in cross 328 × 321. The curve drawn in full is that calculated from the combined observations.

In tables 1 and 2 one may notice an F_3 family, No. (328 × 321)1—7, that seems to have repeated the small parent, *N. Langsdorffii*. This was indeed the case. Several F_2 plants duplicated *N. Langsdorffii* in every feature and two of them were selfed and their progeny grown. Family No. (328 × 321)1—1 from the larger of these two plants as

regards flower size was very uniform, but the flowers were slightly larger than those of the *N. Langsdorffii* strain used, and the plants differed from it slightly in other ways. In short, it could hardly be maintained that the great-grandparent had been duplicated. On the other hand family (328 × 321)1—7 was exactly like a family from a selfed plant of No. 328. I could not find a distinguishing trait by the most minute examination. Figures 6, 7 and 8 show this fact plainly, but since photographs of the other small family would appear to show the same thing, so small are the differences between it and our strain of No. 328, a table of corolla measurements has been introduced in order to demonstrate the matter quantitatively.

Since it is obvious that the use of 3-mm classes in table 1—classes as small as can be treated conveniently in connection with such great size difference—obscures somewhat the true distribution of the corolla length of No. 328, table 5 gives the distribution of the corolla lengths of the

TABLE 5

Frequency distribution of No. 328 and of an F_3 family that bred true to the characters of this species.

Pedigree Number	Class centers in millimeters					Mean	S. D.	C. V.	No.
	19	20	21	22	23				
328, (1911)		3	12	1	2				
328, (1914)	1	9	33	7	1	20.96 ± .06	0.69 ± .05	3.27 ± .21	51
328, total	1	12	45	8	3	21.00 ± .06	0.72 ± .04	3.43 ± .20	69
(328 × 321) 1 — 7 F_3		11	33	6		20.90 ± .05	0.57 ± .04	2.75 ± .19	50

same plants in 1-mm classes. This brings out the wonderful uniformity of the populations of both No. 328 and No. (328 × 321)1—7 and the marvelous similarity between the two families. Furthermore, it shows how similar are two populations of No. 328 grown from the same seed but in different years.

THE RECIPROCAL CROSS

The cross in which No. 321 was used as the mother, was not a true reciprocal of the other in that the same individuals were not used. In fact a different strain of *N. Langsdorffii* known as No. 328—1 was used, which had flowers slightly smaller than No. 328. For this reason as well as that each generation of this cross was grown a year later than the

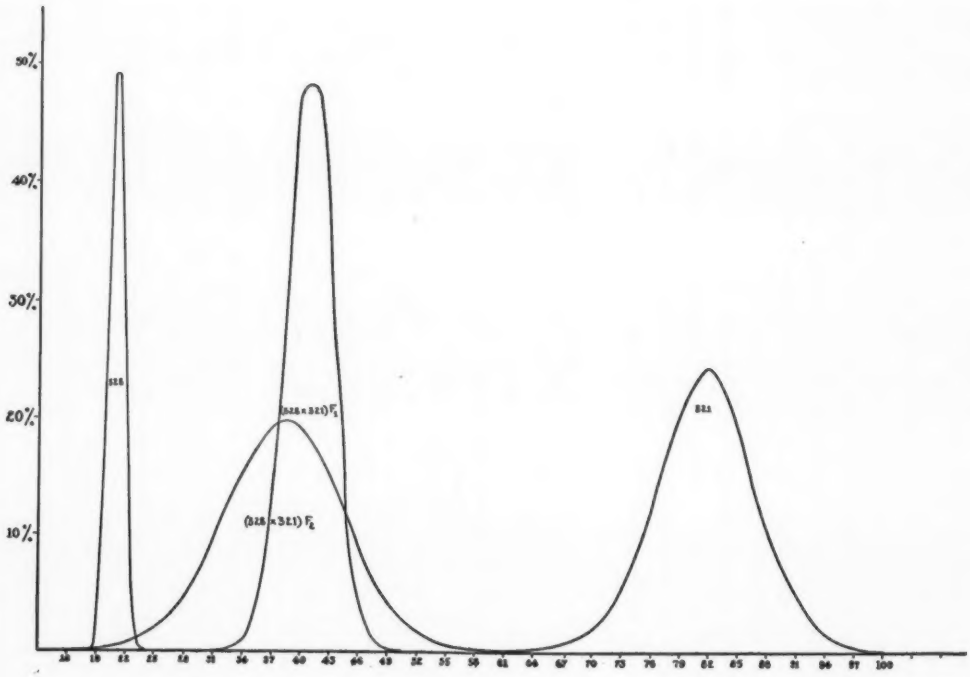


FIGURE 10. The theoretical curves of flower length for population of *N. Langsdorffii* (328), of *N. alata grandiflora* (321), and of the F_1 and F_2 of the cross 328 × 321. The curve of 328 cannot be compared with the others because it is shown in classes of one millimeter instead of classes of three millimeters.

Table 6

Frequency distribution of corolla length in the families of cross 321 × 328.

Pedigree Number	Size in parent	Class centers in millimeters																									No.
		16	19	22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	
328—1, (1910)			26	4																									30
328—1, (1912)		2	20	4																									26
328 – 1, total		2	46	8																									56
321, (1911)																					3	8	10	11	8	5	3	1	49
(321×328—1) F_1										8	21	2																	31
(321×328—1) F_2				1	0	9	16	21	53	25	23	6	6	3															163
(321×328—1)1—1 F_3	22	8	75	18																									101
(321×328—1)1—2 F_3	49								7	17	25	18	12	2															81
(321×328—1)1—3 F_3	51						1	2	4	8	30	31	19	6	1	2	0	1											105
(321×328—1)1—4 F_3	49								6	8	19	15	18	1															67

other, the slight differences between the two can not be attributed to the different way of making the cross. The cross with No. 321 as the mother was more difficult to make but this is probably due to the greater length of the style of No. 321.

Cross 321 × 328 is notable for the extreme uniformity of the first hybrid generation and the great increase in variability in the second hybrid generation, as is shown in tables 6 and 7. *N. Langsdorffii* was again

TABLE 7

Statistical constants for the frequency distributions of corolla length shown in table 6.

Pedigree Number	Size in parent	Mean	S. D.	C. V.	No.
328 — 1, (1910)		19.40 ± .13	1.02 ± .09	5.26 ± .46	30
328 — 1, (1912)		19.23 ± .19	1.42 ± .13	7.38 ± .69	26
328 — 1, total		19.32 ± .11	1.23 ± .08	6.37 ± .41	56
321, (1911)		81.76 ± .49	5.08 ± .35	6.21 ± .42	49
(321 × 328 — 1) F_1		42.42 ± .19	1.60 ± .14	3.77 ± .32	31
(321 × 328 — 1) F_2		37.79 ± .28	5.36 ± .20	14.18 ± .54	163
(321 × 328 — 1) 1 — 1 F_3	22	19.30 ± .10	1.50 ± .07	7.77 ± .37	101
(321 × 328 — 1) 1 — 2 F_3	49	43.63 ± .28	3.73 ± .20	8.55 ± .45	81
(321 × 328 — 1) 1 — 3 F_3	51	45.34 ± .32	4.80 ± .22	10.59 ± .50	105
(321 × 328 — 1) 1 — 4 F_3	49	44.52 ± .32	3.85 ± .22	8.65 ± .50	67

reproduced in F_2 and plant (321 × 328) 1—1 bred true to its characters. There was no nearer approach to No. 321, however, than there was in cross 328 × 321. The cross appeared to be fully fertile and the seeds germinated well though in general not so perfectly as those of the reverse cross. It does not seem as if the slight infertility shown, however, could be the explanation of the failure to reproduce the larger parent.

Again the coefficients of variability of the four F_3 families grown are below that of the F_2 generation. Considering them together with the other later generations previously reported it would seem as if the case for Mendelian inheritance were pretty clearly proven.

CONCLUSION

A fertile cross between two distinct species, *Nicotiana Langsdorffii* and *Nicotiana alata grandiflora*, each uniform in its characters, has been reported here with the following results, no matter which way the cross was made.

1. The F_1 populations are as uniform as the parents.

2. The F_2 generations are nearly three times as variable as the F_1 generations.

3. Individuals reproducing the smaller species were found in the F_2 generation.

4. Certain of these F_2 individuals reproduced *N. Langsdorffii* populations in the F_3 generation.

5. No F_2 individuals reproducing *N. alata grandiflora* were found, but F_3 plants approaching such a type were produced.

6. Galtonian regression occurred, but selected extremes regressed no more than those deviating moderately from the parental mean.

7. Individuals from the same point on the F_2 curve showed different variabilities in F_3.

8. The variabilities of F_3 families were invariably smaller than those of F_2 families.

9. The above conclusions are based upon corolla length measurements but apparently are true for other characters, except that in other characters, *N. alata grandiflora* types were reproduced.

10. Mendelian inheritance of corolla color and pollen color is shown.

11. Mendelian inheritance seems to be the *only* logical interpretation of the other phenomena.

LITERATURE CITED

COMES, O., 1899 Monographie du genre Nicotiana. Naples: Typographie Coöpérative.

DORSEY, M. J., 1915 Pollen sterility in grapes. Jour. Heredity **6**: 243-249.

EAST, E. M., 1916 a Significant accuracy in recording genetic data. Amer. Jour. Bot. **5**: 211-222.

1916 b Studies on size inheritance in Nicotiana. Genetics **1**: 164-176.

EAST, E. M., and HAYES, H. K., 1912 Heterozygosis in evolution and in plant breeding. U. S. D. A. Bur. Plant Ind. Bull. 243, pp. 58.

FOCKE, W. O., 1881 Die Pflanzenmischlinge. 569 pp. Berlin: Borntraeger.

GODRON, D. A., 1863 Des hybrides végétaux considérés au point de vue de leur fécondité et de la perpétuité ou non-perpétuité de leurs caractères. Ann. sci. nat., Bot. Ser. IV, **19**: 135-179.

GOODSPEED, T. H., 1913 On the partial sterility of Nicotiana hybrids made with *N. sylvestris* as a parent. Univ. Cal. Pub. Bot. **5**: 189-198.

LOCK, R. H., 1909 A preliminary survey of species crosses in the genus Nicotiana from the Mendelian standpoint. Ann. Roy. Bot. Gar. Peradeniya **4**: 195-227. Pl. 18-29.

NAUDIN, Ch., 1865 Nouvelles recherches sur l'hybridité dans les végétaux. Nouv. Arch. Mus. Paris **1**: 25-176. Pl. 9.

SOME CORRELATIONS IN SUGAR BEETS

F. S. HARRIS AND J. C. HOGENSON

Utah Experiment Station, Logan, Utah

[Received March 21, 1916]

INTRODUCTORY

The beet sugar industry, amounting to hundreds of millions of dollars every year, is the direct result of scientific breeding done on the sugar beet, which has been developed from a plant with a sugar content too low to permit of the commercial extraction of sugar, up to its present high sugar content. If the industry is to be permanent, it is necessary to maintain in the sugar beet its present high sugar content; and it is highly desirable to increase the percentage of sugar as much as possible. This means that breeding methods will need to be applied to the sugar beet as long as it is used in the manufacture of sugar.

The improving of any plant is necessarily a slow and expensive process. This is particularly true of the sugar beet, since two years are required for the production of seed, and also a vast amount of expensive chemical work is necessary in determining the quality of the plants. It is, therefore, highly desirable to learn as much as possible of the nature of the sugar beet and of the correlation of its various characters in order, if possible, to obtain short cuts in the process of improvement.

At the UTAH EXPERIMENT STATION the selection of sugar beets has been going on for thirteen years. During this time, fairly complete records, including pedigrees, have been kept. These records furnish the data necessary to determine the correlation of characters herein discussed.

LITERATURE

Considerable work has been done on the correlation of different characters in sugar beets; but the results are not entirely uniform and they have led to considerable divergence of opinion.

LASKOWSKY (1893) as a result of experiments in the Wakï district, Russia, concluded that beets of the highest sugar content are produced from the smallest seed balls; also that the individual seeds in these smallest seed balls are highest in fat.

TOWAR (1901) found that the varieties of beets which gave the highest yield contained the lowest percentage of sugar.

MAAS (1906) obtained results which showed that the percentage of sugar within a variety decreased as the weight of the beet increased, but the rate of increase was not the same, so that the total quantity of sugar was more dependent on the quantity than on the quality of beets.

ANDRLIK and URBAN (1908) showed from analyses that abnormally large sugar beets had a characteristically low sugar content.

Later, they in conjunction with BARTOS (ANDRLIK, BARTOS and URBAN 1912) concluded that the weight of sugar beets is more readily modified than the sugar content. They denied the truth of the statement that the greater the weight the less the sugar content except in extreme, and hence rare, cases. From data presented, they conclude that the weight of the beet root shows fluctuating variability in accordance with the QUETELET-GALTON law. Each strain shows its own correlation between root weight and sugar percentage. In only a few unusual cases does it occur that low sugar content accompanies a very unusual root weight, and these cases probably result from abnormalities in the use of plant food.

HARRIS and GORTNER (1913) conclude that the notes presented by ANDRLIK, BARTOS, and URBAN "form a very slender basis for the conclusion that in beets of the same strain there is no negative correlation between weight and sugar content. Nevertheless one must recognize the possibility of the correctness of the conclusion. Should it be valid, the suggestion follows that the negative correlation demonstrated in commercial cultures has a genetic origin, i.e., that strains characterized by large root size are also characterized by low sugar content, and that when these strains are intermingled and intercrossed in field cultures there results a negative correlation between the weight of the individual beet and the sugar content of its juice."

METHODS

The work reported in this paper was done at the UTAH EXPERIMENT STATION in Logan, Utah. It was begun in 1902, when a number of strains of seed were obtained from the U. S. DEPARTMENT OF AGRICULTURE and from local sugar companies. Since that time, selections have been made for high sugar content and yield and for desirable size and shape of beets.

From the beets raised each year samples having a desirable size and

shape were saved and analyzed. The very large and the very small beets were eliminated and only the medium beets saved. This eliminates the extremes which ANDRLIK, BARTOS, and URBAN consider to be the only ones in which a negative correlation between size and sugar content is shown. Certainly if these extremes had been included, the correlation would have been more striking.

The analyses were made from pulp obtained by boring a diagonal hole through each beet. The beets were then siloed over winter in dry sand and in the spring were planted four feet apart each way. During the blossoming period, the tops were covered with mosquito bar to prevent an interchange of pollen by insects. At maturity of the seed, determinations were made of the height of plants, number of stems, average number of leaves on each stem, weight of seed, and number of days from planting to maturity of seed. These data, together with the weight of the mother beet and the percentage sugar it contained, furnish the material for the correlation tables which are here presented.

The method of calculating the coefficient of correlation and the probable error is that explained by EUGENE DAVENPORT in his *"Principles of Breeding,"* pp. 419-472. Where there is a perfect correlation in two characters, the coefficient of correlation is 1, while if there is no correlation the coefficient is zero. As the correlation varies from none to complete, the coefficient varies from 0 to 1. Where the correlation is negative the coefficient is preceded by a minus sign.

CORRELATIONS

1. *Weight of beet and sugar content*

As pointed out in the literature review, there has been considerable controversy over the relation between the size of beets and their sugar content.

Table 1 shows results for 6984 beets which were raised during a number of years. A negative correlation of —.2878 is shown between these two characters, with a probable error of ±.0074. It must be kept in mind that neither the very small nor the very large beets were used. As already pointed out, ANDRLIK, BARTOS, and URBAN (1912) have shown these extremes to give the most obvious relationship between these two characters. It would seem, therefore, that the correlation can be considered as fairly definite though by no means complete. The smaller beets tend to have a higher percentage sugar than the larger ones.

TABLE 1

Correlation of weight of beet with percentage sugar

Weight of beets in kilograms	Percent sucrose. Below 6.5	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	21.5+	
2.1											1							1
2.0				2		2	12	14	11	6	4	2	1					54
1.9							4	4	5	4	5	5	1					28
1.8				1	1	3	3	14	8	16	9	4	2					61
1.7					2	5	7	9	10	13	13	7	6	1				73
1.6			1		1	3	5	19	28	32	22	20	8	2	1			142
1.5					4	3	7	20	32	39	38	29	25	8	1			206
1.4		1		3	2	3	11	31	46	51	38	42	34	13	4			279
1.3		1		4	3	6	20	27	57	55	88	67	53	25	4	1		411
1.2	2	1	2	1	4	11	15	31	60	72	79	69	63	37	19	2	2	470
1.1	3	1	5	2	7	6	20	42	48	88	112	91	116	49	27	5		622
1.0	2	2	2	3	13	14	22	24	65	98	113	133	105	68	32	5		701
.9	5	6	3	5	8	7	18	40	57	96	108	167	138	115	41	10	1	825
.8		1	6	8	13	16	17	26	60	80	81	141	134	105	51	11	1	751
.7	7	1	2	5	9	7	17	24	35	60	84	135	124	65	55	6		636
.6	3	2	2	7	6	8	14	20	21	45	69	97	106	60	42	4	2	508
.5	1				14	4	4	6	23	30	49	90	105	44	43	8	9	430
.4					4	3	5	4	12	28	49	57	85	69	42	16	11	385
.3					3		3	5	8	25	28	35	72	50	31	12	11	283
.2							1	6	3	6	10	15	33	14	14	6	10	118
Total	23	16	23	41	94	101	205	366	589	844	1000	1206	1211	725	407	86	47	6984

Correlation = — .2878 ± .0074.

Mean weight of beets 9328 ± .0297; standard deviation 3.681 ± .0210
Mean percent sucrose 16.1327 ± .0205; standard deviation 2.537 ± .0145

2. *Percentage sugar in mother beet and weight of seed*

An examination of table 2 shows a correlation of .0049 between the percentage sugar in the mother beet and the weight of seed produced. The probable error is ±.0143 which is greater than the correlation. This means that there is no correlation whatever between these characters.

TABLE 2

Correlation of percent sugar in the mother beet with the weight of seed produced.

Weight of seed in kilograms.

Percent sucrose in mothers.	Below .05	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0	1.1	1.15+	
Above 22			1	1	1		1			1				5
22	2	5	3	8	2			2						22
21	5	18	12	12	11	3	3	1						65
20	9	23	41	49	56	17	7	2	6	2		1		213
19	27	39	35	56	71	45	17	12	7	3	1	3	1	317
18	38	56	61	103	142	96	50	16	9	8	6	3	4	592
17	5	17	32	76	97	59	21	13	12	5	7	6	1	351
16	9	4	9	11	15	9	9	17	4	11	9	4	4	115
15	7	9	7	8	12	13	5	3	5	7	6	3	4	89
14	7	15	15	17	12	11	11	8	7	2	3	1	6	115
13	6	10	10	17	15	5	2	2	2	1	1	3	1	75
12	1	10	16	18	12	8					1			66
11		5	12	21	12	5	3	1	1					60
10	2	7	13	17	11	4			1					55
9	1	4	11	10	6	1								33
8	1	9	15	15	12							1		53
Total	120	231	293	439	487	276	129	77	54	40	34	25	21	2226

Correlation = .0049 ± .0143.

Mean weight of seed .3789 ± .03408; standard deviation 2.384 ± .0241
Mean percent sucrose 16.7800 ± .0433; standard deviation 3.029 ± .0306

3. *Height of plant and weight of seed*

Table 3 gives results with 1954 beets, and shows a correlation of .3985 with a probable error of ±.0128. The higher plants, therefore, have more seed than the lower ones.

TABLE 3

Correlation of height of plants with weight of seed produced.

Height of plant in meters.	Weight of seed in kilograms.											
	Below .15	.2	.3	.4	.5	.6	.7	.8	.9	1.0	1.05+	
Above 1.4	6	6	6	15	8	14	14	13	16	9	12	119
1.4	13	13	21	16	17	9	8	8	6	4	5	120
1.3	11	18	37	43	36	18	12	12	5	7	7	206
1.2	66	66	81	99	86	34	18	6	5	2	8	471
1.1	42	43	70	113	63	27	11	8	7	1	3	388
1.0	13	40	62	78	44	17	2	3	2	1		262
.9	42	31	59	43	21	6	4		2			208
.8	26	25	21	13	4	3						92
.7	5	7	7	8	1	1						29
.6	11	3	3	2								19
.5	12	3	3									18
.4	18	1	1	2								22
Total	265	256	371	432	280	129	69	50	43	24	35	1954

Correlation = .3985 ± .0128.

Mean weights of seed .3934 ± .0339; standard deviation 2.222 ± .0240
Mean height of plant 1.1150 ±.0324; standard deviation 2.125 ± .0229

4. *Weight of mother beet and yield of seed*

A comparison of the relative quantity of seed produced by 2168 beets showed that there was a correlation of .3075 ±.0131 between quantity of seed and the weight of the mother beets. That is, large beets on the average yielded more seed than small ones.

TABLE 4

Correlation of weight of mother beet with weight of seed produced.

Weight of beets in kilograms. (Above)	Weight of seed in kilograms. Below .05	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0	1.1	1.15 +	
2.0			1		2	1						1		5
2.0							2							2
1.9					1	1		1	1			1	1	6
1.8			2			2		1					1	6
1.7	1		1	2	3	3	1		2		1	1	2	17
1.6	1	1	5	2	6	5	2	2		2	1	1	1	29
1.5	2		3	4	8	6	9	1	1		1			41
1.4	1	2	1	3	6	11	3	3	3	4		1	1	39
1.3	1	3	2	4	22	15	14	4	4	2	5	1	2	79
1.2	2	3	10	12	28	18	11	13	9	3	5	1	6	121
1.1	1	3	10	17	29	20	19	11	11	12	3	4	3	143
1.0	10	11	8	25	41	38	23	18	15	6	6	8	4	213
.9	5	13	22	39	51	54	38	18	16	12	9	7	3	287
.8	7	17	20	43	70	52	24	25	18	12	6	3	4	301
.7	16	12	27	35	52	49	27	17	14	6	6	2	1	264
.6	14	35	25	40	50	32	25	11	5	9	6	2	3	257
.5	15	25	20	31	26	27	7	3	4	3	2	1		164
.4	20	22	21	15	12	2	1	1		2		1		97
.3	15	28	12	8	3	2	1							69
.2	4	5	5	4	4	3				1	1	1		28
Total	115	180	195	284	414	341	207	134	103	75	51	37	32	2168

Correlation = .3075 ± .0131.

Mean weight of beets .8448 ± .0462; standard deviation 3.189 ± .0327
Mean weight of seed .4503 ± .0388; standard deviation 2.680 ± .0274

5. *Number of stems and yield of seed*

Table 5 shows a correlation of .2771, with probable error of ±.0133, between the number of stems on each plant and the weight of seed. As would be expected, the plants having most stems produce the largest quantity of seed.

TABLE 5

Correlation of number of stems per plant with the weight of seed produced

Number of stems per plant.	Weight of seed in kilograms.													
	Below .05	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0	1.1	1.15+	
Above 34			3	8	12	6	5		1	1	1	1	1	39
32-34		1	1	2	3	3	2	3	1	1		1	1	19
30-32		1	3	3	6	5			2		1	2		23
28-30			2	3	7	2	3	2	1	1		1		22
26-28		1	3	7	9	2	3		1	1	1			28
24-26	1		3	8	16	8	3	1	1		1			42
22-24		3	6	18	19	12	3	3			2	1	3	70
20-22		2	9	21	18	15	6	2	2	2	1	1	1	80
18-20		4	21	31	35	20	8	5	2	4	1	2		133
16-18	5	13	25	34	42	23	8	5		5	5	2		167
14-16	2	14	28	55	70	41	20	9	8	6	7	6	3	269
12-14	6	25	36	59	86	48	22	11	7	6	4	3	3	316
10-12	8	25	36	72	81	40	21	13	12	5		3	4	320
8-10	10	35	47	63	36	27	10	14	7	4	6	1	3	263
6-8	21	28	41	27	20	7	6	10	3	5	2	1	1	172
4-6	21	31	23	24	10	4	1		4		2		1	121
2-4	23	22	13	3	6	3	1		1					72
1-2	26	15	4	2	2									49
	123	220	304	440	478	266	122	78	53	41	34	25	21	Total 2205

Correlation = .2771 ± .0133.

Mean weight of seed .3777 ± .0344; standard deviation 2.392 ± .0243
Mean number of stems 14.3642 ± .0990; standard deviation 6.892 ± .070

6. *Number of days to mature and yield of seed*

The time required to mature seed varied from 88 to 148 days, most of the beets maturing in about 105 days. Table 6, including 1724 beets, shows that the yield of seed was somewhat higher in the beets requiring the longest time to mature. The coefficient of correlation between these characters was .1954 ±.0156.

TABLE 6

Correlation of number of days to mature with the weight of seed produced.

Days from planting to maturity of seed.	Weight of seed in kilograms.													
	Below .05	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0	1.1	1.15+	
Above 148	1	1												2
144-148		4	1									1		6
140-144	1	4	3		1									9
136-140	6	6	1		3	1	2		1	1				21
132-136	1	1	2	3	5	1	1	1	1	2			1	19
128-132	7	2	4	2	1	7	8	6	9	8	6	2	2	64
124-128	10	12	9	11	9	3	4	12	8	5	8	4	8	103
120-124	2	7	4	9	1	1	7	7	2	6	3	1		50
116-120	2	5	21	33	23	7	5	11	5	7	2	5	6	132
112-116	4	10	14	14	11	6	6	15	7	2	7	6	2	104
108-112	2	20	20	16	19	9	12	9	9	5	3	3	1	128
104-108	15	30	51	74	84	72	19	9	10	4	3	2	1	374
100-104	10	11	28	67	116	61	27	7	2					329
96-100	5	19	19	39	67	41	16							206
92-96	2	5	12	34	37	22	4			1	1			118
88-92	7	2	9	17	15	3								53
88		4	2											6
	75	139	202	321	392	234	111	77	54	41	33	24	21	Total 1724

Correlation = .1954 ± .0156.

Mean weight of seed .4116 ± .0401; standard deviation 2.471 ± .0284
Mean days to mature 110.2230 ± .1848; standard deviation 11.372 ± .131

7. *Number of leaves and yield of seed*

TABLE 7

Correlation of the number of leaves on each stem with the weight of seed produced.

Weight of seed in kilograms.

Number of leaves on each stalk.	Below .05	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0	1.1	1.15+	
Above 29	1	1	3	8	17	7	3							40
29			1	2	3	2	6	1						15
28			1	2	3	4	1							11
27			1	3	8	2	2							16
26	1		2	6	9	1	2							21
25		1	5	10	12	2								30
24		4		8	15	6	2	2						37
23			2	8	16	7	3							36
22		1	7	8	16	18	3	2					1	56
21		4	5	14	12	9	1		1	1				47
20	5	19	21	19	18	12	3	1	1	1				100
19	17	40	31	31	22	16	3	2	1					163
18	15	26	27	25	33	17	6		1	2		1		153
17	14	27	19	16	25	21	5	1	2	2			1	133
16	3	15	27	41	40	14	7	5	3	2	3	1	3	164
15	10	17	23	43	43	20	14	4	2	5	6	2	3	192
14	6	11	24	41	43	26	14	11	2	5	2	3		188
13	3	4	12	24	27	19	7	9	6	4	2	5		122
12	5	15	26	50	37	22	16	10	12	3	7	3	6	212
11	6	2	16	20	26	16	6	9	4	5	3	2	1	116
10	8	12	28	34	29	10	12	12	11	6	3	5	3	173
9	3	5	4	3	6	7	4	6	7	5	4	3	2	59
9	23	7	7	10	9	5	1	2	2		3		1	70
	120	211	292	426	469	263	121	77	55	41	33	25	21	Total 2154

Correlation = .1217 ± .0143.

Mean weight of seed .3805 ± .0349; standard deviation 2.403 ± .0247
Mean number of leaves per stem 15.8222 ± .0710; standard deviation 4.886 ± .0502

While there seems to be some correlation between the number of leaves on each stem and the quantity of seed produced by a beet, it is not so great as between some of the other characters already compared. Table 7 shows this correlation to be .1217 ±.0143.

8. *Number of days to mature and height of plants*

On comparing the days to mature with the height of plants, it is found in table 8 that a relationship exists, but this relation is not great, the correlation being .1748 ±.0163. The taller plants require a slightly longer time to mature than shorter ones.

TABLE 8

Correlation of the days to mature with the height of plants.

Days from planting to maturity of seed.	Height of plant in meters.													
	Below .35	.4	.5	.6	.7	.8	.9	1.0	1.1	1.2	1.3	1.4	1.45+	
Above 144			2	2			2			10				16
140-144			1	3		8	4			3				19
136-140			1	2	1	5	4		3	3	3		2	24
132-136				1	1	1	3	2	2	1	1	1	3	16
128-132			1			3	1	1	4	14	12	9	25	70
124-128	1	1	4	2	1	1	8	6	16	23	14	8	22	107
120-124			4			1	6	3	7	11	5	9	11	57
116-120		1	2	1	1	2	12	17	20	16	12	9	15	108
112-116			1			1	9	7	14	15	8	2		57
108-112				1	3	6	13	10	20	22	4	4	2	85
104-108	3		4	5	1	9	34	45	64	98	46	17	12	338
100-104	1	3	2	4	5	15	25	58	93	84	27	9	3	329
96-100	1	1	3	2	8	9	34	32	39	58	11	8	1	207
92-96	1	1		3	5	15	21	23	20	18	5	2	2	116
88-92	4	1	2	1	4	7	12	5	9	3	5			53
88					2	2	1						1	6
	11	8	27	27	32	85	189	209	311	379	153	78	99	Total 1608

Correlation = .1748 ± .0163.

Mean height of plant 1.0940 ± .0381; standard deviation 2.266 ± .0269
Mean days to mature 110.5100 ± .2084; standard deviation 12.384 ± .147

Days from planting to maturity of seed.

9. *Percentage sugar in mother beet and time to mature seed*

Table 9 shows a negative correlation of —.1292 with a probable error of ±.0155 between the sugar content and the date of maturity of seed. That is, the higher the sugar content of the mother beet the less time is required to mature seed. The correlation is low however.

TABLE 9

Correlation of the days to mature seed with the percentage sucrose in the mother beet.

Days from planting to maturity of seed.	Percentage sucrose in mother beets.															
	Below 8.5	9	10	11	12	13	14	15	16	17	18	19	20	21	21.5+	
Above 148											4			1	2	7
144-148									1		1		1	3	3	9
140-144						1	1		2		4	5	5	3		21
136-140							2		4	1	3	4	7	1		22
132-136							3	2	3	3	3	2	2	1	1	20
128-132			1			4	8	6	10	8	14	15	5	2	1	74
124-128					1	7	15	14	15	11	25	12	14	4	4	122
120-124						1	4	6	8	5	15	15	4	1	1	60
116-120	5	8	7	5	6	8	16	11	17	18	14	15	7	1	1	139
112-116	1			1			2	8	8	14	23	16	20	7	5	105
108-112	4		4	3	3	2	5	3	7	19	41	18	9	8	2	128
104-108	1	1	2	2	6	7	12	10	12	86	139	63	42	14	3	400
100-104			2	3	1	5	11	6	9	85	108	61	38	6	6	341
96-100			1	1	5	3		3	3	36	79	46	26	8	1	212
92-96							1	2	1	13	49	26	17	2	1	112
88-92			1	2						6	16	14	8	3	2	52
88						1					1	2	1	1		6
	11	9	18	17	22	39	80	71	100	305	539	314	206	66	33	Total 1830

Correlation = — .1292 ± .0155.

Mean percent sucrose in mother 17.5831 ± .0371; standard deviation 2.3548 ± .0262
Mean days to mature 111.0200 ± .1892; standard deviation 12.384 ± .134

10. *Sugar of mother beet and leaves on each stalk*

TABLE 10

Correlation of the number of leaves on each stalk with the percentage sugar in the mother beet.

Percentage sucrose in mother beets.

Number of leaves on each stalk.	Below 8.5	9	10	11	12	13	14	15	16	17	18	19	20	21	21.5 +	
30 and more				1	1		2	1	1	11	15	6	3		1	42
29								1		3	6	4		1		15
28										5	3	2	1			11
27									1	3	9	1	2			16
26					1	1			2	6	7	2	1		1	21
25	1					1	2		1	8	8	7	1			29
24										9	19	4	3			35
23				1						4	21	8	1	1		36
22			1				2	1	1	12	29	6	3	1	1	57
21				1					1	7	20	12	8		2	51
20						2	2	1	4	14	35	26	19	3	2	108
19			1				1	1	1	10	67	57	31	12	8	189
18	1			3	2		1	4	3	17	52	32	21	8	1	145
17	1				1	2	2	1	4	26	46	31	17	10	3	144
16	7	6	7	8	12	10	11	9	5	24	24	18	12	5	2	160
15	7	6	10	9	9	9	12	12	14	28	38	20	14	1	3	192
14	10	5	9	7	6	8	11	9	9	21	38	23	18	6	3	183
13	4	3	6	4	2	6	7	4	8	30	22	18	10	2		126
12	8	2	7	9	10	9	20	16	17	30	52	15	10	1		206
11	1	4	2	5	4	4	5	5	10	23	23	12	14	4	3	119
10	9	5	8	9	10	12	12	11	16	32	26	9	12	3	1	175
9	1		1	1	1	3	6	5	7	9	13	3	8	2	3	63
8 and less	2	2	2	1	3	3	11	7	5	9	12	12	15	8	1	93
	52	33	54	59	62	70	107	88	110	341	585	328	224	68	35	Total 2216

Correlation = .2484 ± .0134.

Mean percent sucrose 16.8642 ± .0441; standard deviation 3.080 ± .0312

Mean number of leaves per stem 15.7897 ± .0706; standard deviation 4.927 ± .0499

Table 10 shows a variation in the number of leaves on each stalk from about 8 to about 30. The correlation of this character with the sugar content of the mother is shown to be .2484 ±.0134. This coefficient of correlation seems sufficiently large to justify the statement that as the mother beet increases in sugar there is a tendency for more leaves to grow on each seed stalk.

SUMMARY

The tables which have been presented show the following correlations of characters in sugar beets:

1. Weight of beet with percentage sugar = —.2878 ±.0074.
2. Percentage sugar in the mother beet with quantity of seed produced = .0049 ±.0143.
3. Height of plants with weight of seed produced = .3985 ±.0128.
4. Weight of mother with quantity of seed produced = .3075 ±.0131.
5. Number of stems on each plant with the quantity of seed produced = .2771 ±.0133.
6. Number of days to mature with quantity of seed produced = .1954 ±.0156.
7. Number of leaves on each stalk with quantity of seed produced = .1217 ±.0143.
8. Days to mature with height of plant = .1748 ±.0163.
9. Percentage of sucrose in mother beet with days to mature seed = —.1292 ±.0155.
10. Percentage of sucrose in mother beet with number of leaves on each stalk = .2484 ±.0134.

LITERATURE CITED

ANDRLIK, K., and URBAN, J., 1908 Abnormally large beets. Zeitschr. Zuckerindus: Böhmen **32**: 493-496. Abstract Exp. Sta. Record **20**: 747.

ANDRLIK, K., BARTOS, V., and URBAN, J., 1912 The mutual relations of weight and sugar content in *Beta vulgaris saccharifera*. Zeitschr. Zuckerindus. Böhmen **36**: 193-210. Abstract Exp. Sta. Record **27**: 642.

HARRIS, J. A., and GORTNER, R. A., 1913 On the relationship between the weight of the sugar beet and the composition of its juice. Jour. Indus. Engin. Chem. **5**: 192-195, and Biochem. Bull. **2**: 524.529.

LASKOWSKY, N., 1893 The relation between the size and composition of beet seeds and the size and composition of the roots produced by them. Landbote **14**: 600. Abstract Exp. Sta. Record **5**: 335.

MAAS, H., 1906 Investigations on correlation in fodder beets. Landw. Jahrb. **35**: 84-113.

TOWAR, J. D., 1901 The influence of size of beets on sugar content. Michigan Exp. Sta. Bull. 197, pp. 140-144.

NOCHMALS UEBER DIE MEROGONIE DER OENOTHERABASTARDE

RICHARD GOLDSCHMIDT

Kaiser Wilhelm Institut für Biologie, Berlin

[Eingegangen am 20. April 1916]

Im Jahre 1912 veröffentlichte ich eine vorläufige Mitteilung, in der ich zeigte, dass das merkwürdige Erbverhalten der DE VRIES'schen doppeltreziproken Oenotherabastarde restlos durch die Annahme einer Merogonie dieser Bastarde erklärt wird. Eine vorläufige Untersuchung der Tatsachen schien mir zu beweisen, dass sich die Merogonie auch morphologisch zeigen lässt. RENNER, der auf meinen Wunsch hin es unternommen hatte, den Gegenstand weiter zu untersuchen, kam aber zu dem Resultat, dass ich mich getäuscht habe, und bei diesen Bastarden die cytologischen Vorgänge der Befruchtung und Entwicklung normal verlaufen. Da ich nicht glauben konnte, das ich mich derart geirrt habe, beschloss ich, bei erster Gelegenheit den Gegenstand nochmals gründlich zu untersuchen. Das ist nunmehr geschehen und es sei sogleich gesagt, dass ich mich leider überzeugen musste, dass RENNER in den meisten Punkten im Recht ist. Es scheint, dass die suggestive Kraft der Merogonieidee meinen Blick bei der ersten Untersuchung trübte, und es bleibt mir nichts übrig als es offen zuzugeben. Die Neuuntersuchung hat mich nun aber mit neuen Bildern bekannt gemacht, die es mir wahrscheinlich machen, dass doch eine Merogonie stattfindet, und so komme ich nochmals auf den Gegenstand zurück.

Von BAUR, RENNER, DAVIS u. A. ist an den DE VRIES'schen Resultaten Kritik geübt worden. Wäre sie berechtigt, dann hätte eine cytologische Untersuchung keinen Sinn. Denn wenn das Erbverhalten nicht den DE VRIES'schen Angaben entspricht, dann kann es auch nicht mit Merogonie erklärt werden. Mir scheinen allerdings die Einwände wenig stichhaltig zu sein, da sie einmal den noch unbekannten Umfang der plasmatischen Vererbung nicht berücksichtigen (z. B. der Hinweis darauf, dass die F_1 Bastarde nicht rein väterlich sind), oder vergessen,

dass die nicht in reinen Linien gezogenen Eltern nicht völlig homozygot sein müssen (der Einwand, dass F_2 nicht völlig konstant sei). Aber trotzdem erschien mir eine Neuuntersuchung nur dann wünschenswert, wenn sie sich auf ein genetisch einwandfreies Material beziehen konnte. Dies wurde mir nun durch die grosse Liebenswürdigkeit von G. H. SHULL ermöglicht, der sich der Mühe unterzog, in seinem Pedigreematerial die nötigen Kreuzungen auszuführen und mir ein sehr umfangreiches Material zu beschaffen. Es ist mir eine angenehme Pflicht ihm dafür herzlichst zu danken, und ebenso auch Dr. METZ, der die Conservierung nach meinen Angaben ausführte.

Dr. SHULL ermächtigt mich die folgenden brieflichen Angaben über die betreffenden Bastarde zu zitieren:

"The two species of which I sent you pure-bred material and reciprocal crosses were *Oenothera atrovirens* SHULL and BARTLETT and *Oe. venosa* SHULL and BARTLETT. You will find these two species fully described taxonomically, with illustrations, in the AMERICAN JOURNAL OF BOTANY, **1**: 239-242, May, 1914. Unfortunately I cannot refer you to any published account of the hybridization experiments, involving these two species, but the facts are briefly as follows: The hybridization phenomena in these two species represent a typical case of the kind described by DE VRIES as 'double reciprocal hybrids.' Specifically the F_1 progenies are uniform, but quite distinct from one another. With respect to the type of branching and form of buds the F_1 hybrids are strongly patroclinous, but in pigmentation they are just as strongly matroclinous. The F_1 hybrids breed true to their hybrid characters. If we let *Oe. atrovirens* be represented by a, *Oe. venosa* by v, the F_1 hybrids produced by a $\times$ v by N, and those produced by v $\times$ a by O, the total results of my extensive genetical experiments with these two species may be described as follows:

$$
\begin{aligned}
a \times \text{self} &= a \\
v \times \text{self} &= v \\
N \times \text{self} &= N \\
O \times \text{self} &= O \\
a \times N &= N \\
a \times O &= a \\
v \times N &= v \\
v \times O &= O \\
N \times O &= a \\
O \times N &= v
\end{aligned}
$$

"No new forms appeared in the second generation of this cross, and the identity of the several types as indicated by the above symbols was clear enough to be beyond question.

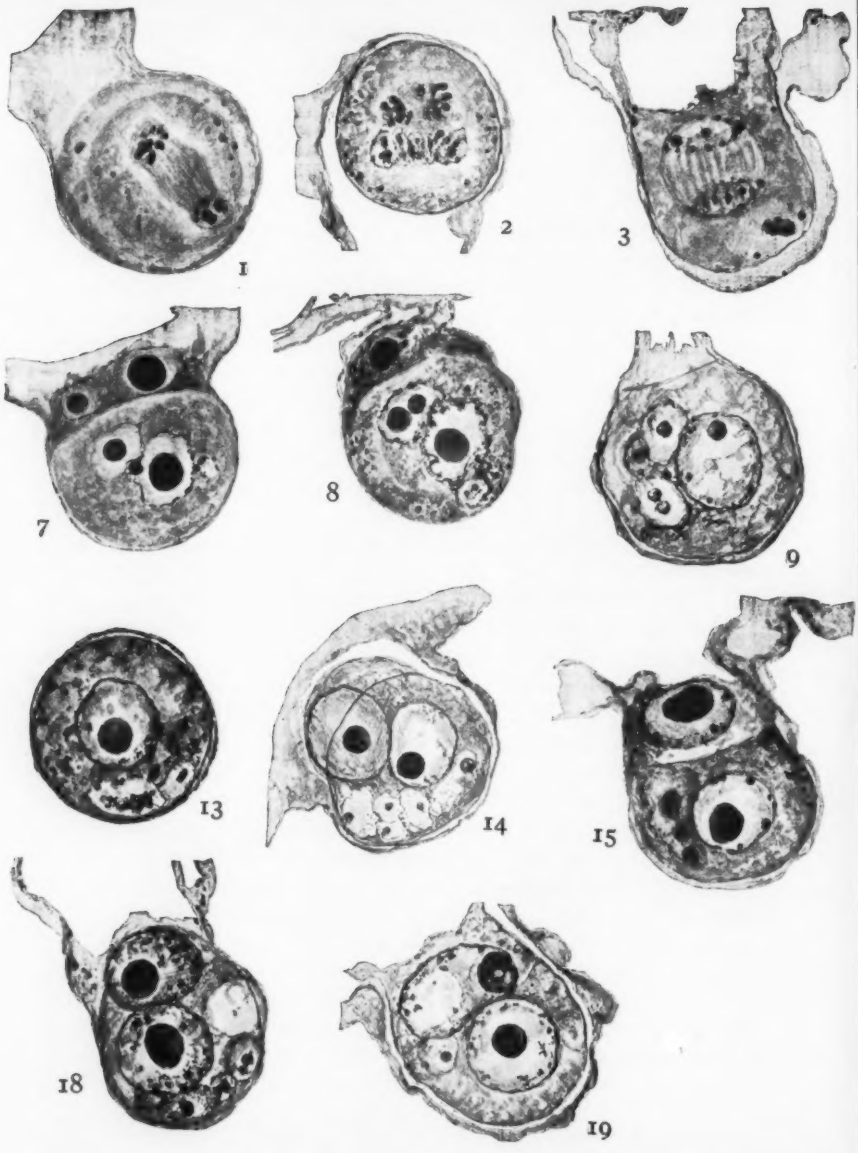
"I should add that your material was all guarded, castrated and crossed by my own hands with the precautions I observe in all my genetical work."

Es kann somit keinem Zweifel unterliegen, dass mein Material sich auf genetisch einwandfreie doppeltreziproke Bastarde bezieht. Ich habe

nun die ersten Vorgänge im befruchteten Ei nochmals verfolgt und mich davon überzeugen müssen, dass sie völlig normal verlaufen. Es ist mir jetzt auch gelungen, eine Aequatorialplatte der 1. Teilung nach der Befruchtung von der Fläche gesehen zu finden und es kann an der diploiden Chromosomenzahl kein Zweifel sein. Bei der weiteren Untersuchung junger Stadien fand ich aber in der ersten Teilung des Embryos Besonderheiten, die es mir sehr wahrscheinlich machen, dass hier eine Chromatinelimination stattfindet, die denn im Endeffekt das gleiche wäre wie eine Merogonie. Trotzdem sämtliche Stadien der kritischen Epoche, die sich in den Präparaten fanden, Andeutungen des Vorganges aufwiesen, muss ich doch zugeben, dass sie nicht als definitiv beweiskräftig betrachtet werden können. Bei der Wichtigkeit des Gegenstandes wäre es wünschenswert, dass mindestens 100 mal das entscheidende Stadium, die späte Telophase der 1. Teilung beobachtet würde. Vielleicht lässt sich das einmal später ausführen, bis dahin kann man nur sagen, dass ein starker Verdacht besteht, dass die Merogoniehypothese doch das Richtige trifft, obwohl sie durch meinen früheren zu raschen Optimismus jetzt diskreditiert erscheint.

Die Tatsachen sind die Folgenden: nach der Befruchtung verläuft alles völlig normal bis zur Telophase der 1. Teilung. In der späten Telophase zeigt sich nun eine deutliche Verschiedenheit zwischen den beiden Polen. An dem dem Suspensor zuliegenden Pol, der der späteren Fusszelle entspricht, gehen alle Vorgänge normal weiter; an dem Embryopol dagegen ordnen sich die Chromosomen in zwei distinkte Gruppen. Fig. 1 (Tafel 4) zeigt das jüngste derartige Stadium.[1] Eine Gruppe zusammengeklumpter Chromosomen, von einem hellen Hof umgeben, liegt hoch im Präparat, darunter eine zweite Gruppe noch isolierter Chromosomen. Ich möchte dies als Gonomerie deuten. Fig. 2 zeigt ein etwas späteres Stadium. Es ist schräg vom Eipol hergesehen, und die beiden in die Figur eingezeichneten Chromatingruppen liegen somit übereinander in verschiedenen Ebenen. Unten ist der Fusszellenpol sichtbar und hier haben sich die Chromosomen bereits in eine Gruppe typischer Karyomeriten umgewandelt. Am Eipol aber sieht man die in der Entwicklung noch nicht so weit vorgeschrittenen Chromosomen in zwei distinkten Gruppen. Eine genaue Zählung ist aber nicht mehr möglich. In Fig. 3 haben wir nun eine späte Telophase. Die Differenz zwischen dem Eizellpol und dem Fusszellpol ist wieder deutlich. An letzterem ist der Kern schon fast aufgebaut, ersterer zeigt noch die Gruppe von Karyo-

[1] Sämmtliche Figuren wurden von Frl. LINA KRAUSE gezeichnet.


1
2
3
7
8
9
13
14
15
18
19

4

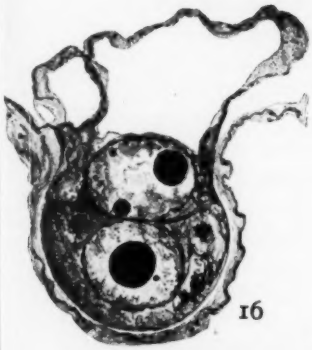
5

6

10

11

12


16

17

20

22

21

meriten intakt. Daneben aber liegt im Plasma in einem hellen Hof eine zusammengeklumpte Chromatinmasse. Noch etwas älter ist Fig. 4 Die Scheidewand ist gebildet und der Fusszellenkern fast fertig. Der Embryokern ist aber noch klein und undeutlich und über ihm im Plasma liegt eine Gruppe chromatischer Kügelchen. Ein etwas späteres Stadium in dem aber die Scheidewand nicht deutlich ist, giebt Fig. 5 wieder. Neben dem Embryokern liegt wieder die chromatische Masse in einem hellen Raum.

Es erscheint mir nun nicht unwahrscheinlich, dass diese Bilder folgendermassen zu deuten sind: In der Telophase tritt eine Trennung der väterlichen und mütterlichen Kernsubstanzen ein, wie dies bekanntlich ja auch bei gewissen von HERBST untersuchten Seeigelbastarden stattfindet. Und dann wird bei der Kernrekonstruktion das mütterliche Chromatin dem Untergang geweiht, wofür auch bis zu einem gewissen Grad Beispiele bei den Seeigelbastarden vorliegen. Der Vorgang findet nur in der Embryozelle, nicht in der Fusszelle statt, was uns im Hinblick auf bekannte Vorgänge des Zellenlebens (Diminution von Ascaris) nicht so absonderlich erscheint. Die Merogonie dieser Bastarde wäre also eigentlich nichts anders als die wohlbekannte (BALTZER, HERBST) Chromatinelimination patrokliner und matrokliner Seeigelbastarde, eine Lösung die ich, wie in meiner ersten Mitteilung schon gesagt, von Anfang an erwartet hatte, von der ich aber durch die falsch interpretierten Stadien mit dem degenerierenden Kern abgelenkt wurde.

Für die Wahrscheinlichkeit dass wir uns hier auf der richtigen Spur befinden, sprechen nun zahlreiche Bilder, die sich in den jungen Zweizellstadien finden. So findet man häufig in der Embryozelle den frischrekonstruierten Kern doppelt, woran sich dann Bilder anreihen, in denen neben dem Kern Gebilde liegen, die sich als die Reste der in Auflösung begriffenen Kernhälfte deuten lassen. Fig. 6 zeigt ein junges Stadium in dem die Scheidewand noch undeutlich ist. Der Kern besteht aus zwei übereinanderliegenden Bläschen. In Fig. 7 und 8 haben wir zwei schräg geschnittene Embryonen in denen die Fusszelle nur angeschnitten ist. In der Embryozelle liegen zwei unregelmässig geformte Kernhälften. Daran schliessen sich nun die zahlreichen Bilder, in denen in jüngeren Zweizellstadien neben dem Kern Bildungen liegen, die man leicht als die zu Grunde gehenden Reste der einen Kernhälfte ansprechen könnte. Fig. 9—20 stellen solche Bilder dar, die sich im Wesentlichen selbst erklären. Wo die Fusszelle nicht sichtbar ist, liegt entweder ein Schrägschnitt oder Querschnitt vor, oder der Embryo ist in einem dicken

Schnitt vom Pol gesehen und nur die Ebene der Embryozelle gezeichnet. Es sei dazu eigens bemerkt, dass von irgendwelchen Degenerationen nicht die Rede sein kann, da ich in den späteren Stadien—im Gegensatz zu dem früheren Material—nicht ein einziges Stadium abnormer Entwicklung fand. Die in Frage kommenden Bildungen bestehen in mehr oder weniger scharf begrenzten, mehr oder weniger kernartigen Vakuolen in denen sich chromatische Reste verschiedener Art finden, die um so undeutlicher werden, je älter das Stadium. In diesen Bildern wird auch sehr oft eine beträchtliche Grössenverschiedenheit zwischen dem Embryo- und Fusszellenkern festgestellt, indem ersterer wesentlich kleiner ist. Es ist dies deutlich in Fig. 11, wo die Umrisse des Fusszellenkernes neben dem vom Pol gesehenen Embryo gezeichnet sind, wobei auch die Grössendifferenz der beiden auffällt.[2] Dasselbe zeigt Fig. 14 und besonders 19 und wurde in etwa der Hälfte der zahlreichen untersuchten zweizelligen Embryonen festgestellt. Es ist besonders beachtenswert, weil der Embryokern sich vor dem Fusszellkern wieder teilt und wenn sein Teilungswachstum eingetreten ist, viel grösser als der letztere wird.

Diese Tatsachen und ihre mutmassliche Deutung erhalten nun noch eine weitere Stütze durch folgenden Befund. In der zweiten Zellteilung, die die Embryonenzelle in zwei Halbkugeln zerlegt, findet sich wieder die diploide Chromosomenzahl. In einem in Fig. 21 wiedergegebenen Fall, lag nun ausserhalb der Spindel ein grosses Chromosom ganz isoliert. Es hatte aber die Grösse der Chromosomen der 1. Teilung oder war sogar grösser. Der Gedanke ist naheliegend, besonders im Hinblick auf ähnliche Fälle bei den Seeigelbastarden,—das hier ein mütterliches Chromosom nachträglich eliminiert wird, das bei der ersten Elimination nicht entfernt worden war. Tatsächlich fand sich denn noch ein Embryo kurz nach dieser Teilung mit einem eliminierten Klumpen Chromatin neben dem einen Kerne. Er ist (im Querschnitt der die Fusszelle nicht zeigt) in Fig. 22 abgebildet.

Die berichteten Tatsachen sind nun zweifellos, obwohl an einem recht umfangreichen Material gewonnen, noch nicht genügend, um die Elimination des mütterlichen Chromatins und somit die Merogonie mit Sicherheit zu erweisen. Die Entscheidung muss somit weiterer Untersuchung überlassen bleiben. Bis dahin kann aber die Merogonie der doppeltrezi-

[2] Es sei hier bemerkt, dass der Nucleolus ein Chromatinnucleolus ist und in der Prophase an dem Aufbau der Chromosomen teil nimmt. Ich habe selten schönere Bilder dieses Vorgangs gesehen als bei diesen Embryonen.

proken Bastarde, die ja die genetischen Erscheinungen restlos erklären würde, keinesfalls als abgetan betrachten werden.

OSBORN ZOOLOGICAL LABORATORY,
New Haven, Conn.
Im März, 1916

ZITIERTE LITERATUR

GOLDSCHMIDT, R., 1912 Die Merogonie der Oenotherabastarde und die doppeltreziproken Bastarde von de Vries. Arch. Zellf. **9**: 331-344.

RENNER, O., 1915 Befruchtung und Embryobildung bei *Oenothera Lamarckiana* und einigen verwandten Arten. Flora N. F. **7**: 115-150.

LINKAGE IN *PRIMULA SINENSIS*

EDGAR ALTENBURG

[Received May 24, 1916]

Primula Sinensis is one of the few plants in which linkage between several pairs of Mendelian factors is known.[1] The work of GREGORY (1911 a, b) has indicated a relationship between these factors essentially similar to that previously established especially well in *Drosophila ampelophila*.[2] No such large numbers of individuals, however, were studied by GREGORY as are customary with the animal workers using such a short-cycled and easily handled type as the pomace fly. It seemed desirable, therefore, to obtain more extensive figures on a form other than Drosophila, in order to have sufficient data on which to base general conclusions on this most recent and important phase of genetics, —the mathematical relationships between linked factors.

Two pairs of factors are said to be linked, if, in an individual heterozygous for them, the parental combinations occur more often than the recombinations (or new combinations). Thus, when two germ-cells unite, one containing the factors for long style and red flower color, and the other those for short style and magenta flower color, an individual is formed most of the gametes of which will contain the parental combinations of factors, namely, some gametes with the factors for long style and red flower, and others with the factors for short style and magenta flower; but a smaller proportion of the gametes will contain the new combinations of factors, namely, gametes with the factors for long style and magenta flower, and others with the factors for short style and red flower. In MENDEL'S experiments involving two pairs of factors, the combination and the recombination classes of gametes were equally numerous; such factors are said to show random segregation. The term "crossover" has been used to apply to new combinations between linked factors, not to the random segregation described by MENDEL.

[1] A group of five pairs of linked factors is known in this plant.

[2] C. B. BRIDGES (1914) has interpreted GREGORY'S results on the chromosome hypothesis.

Investigators in this field have found themselves divided into two camps,—those who adhere to the original reduplication hypothesis of BATESON, and those who find in the chromosomes and their behavior the basis of all Mendelian heredity. It should be a simple matter to test experimentally the hypothesis which states that the members of linked pairs of allelomorphic factors undergo so-called vegetative reductions early in the development of an individual, and that the cells containing certain haploid combinations of factors thus produced (corresponding to the gametic combinations) undergo more rapid division than cells with other combinations, the end result being the production of more gametes of certain classes (the original parental combinations) than those of other classes (the recombination classes). An attempt to test this view will be described later.

The parent plants in my experiments were grown from seed sent to me by R. P. GREGORY, to whom I wish to express my sincerest thanks. One of the parents had the three linked factors, long style (l), red flower (r), and red stigma (s). The other had the three allelomorphic dominants, short style (L), magenta flower (R), green stigma (S).[3] Plants with these characters have been adequately described by GREGORY (1911 a). The F_1, $\frac{l\ r\ s}{L\ R\ S}$, was back-crossed[4] to the triple recessive, $\frac{l\ r\ s}{l\ r\ s}$.

Seeds for the F_1 plants used in the back-cross were planted in the greenhouse during the summer of 1914. The plants started to bloom about the middle of December. The pollinations were made during December, 1914, and January and February, 1915. During these months there were few or no pollen-carrying insects in the greenhouse. The long-styled, red-flowered, red-stigma plants were used in most cases as the female. Examination of many of these long-styled flowers showed

[3] The latter plant appeared pale pink, however, because it differed from the normal magenta in a pair of allelomorphs other than the pair by which normal magenta differs from the red; the pale pink factor is recessive to its allelomorph in the normal magenta (which allelomorph is also possessed by the red).

The pale pink parent was heterozygous for short and long style. I rejected the long-styled F_1 plants.

[4] This method of crossing is very desirable because the factors possessed by the hybrid gametes are shown directly by the character of the resulting offspring, since the other parent introduces no dominants to obscure the factors introduced by the heterozygote. Thus the heterozygote $Ll \times ll$ gives one-half Ll (short style) and one-half ll (long style), the gametes of Ll also being one-half short, and one-half long, style.

that they rarely, if ever, set seed without hand pollination. Moreover, the anthers of these plants did not develop properly during these months, being small, brown and hard, and sterile; they also failed to open. Plants used as female parents in crosses were nevertheless emasculated and sacked before the flowers opened, except in a few instances which were recorded. Plants used as male parents were also sacked before the flowers opened. The sterile anthers of long-styled female plants were removed generally just before pollination or several days previously. This was done by tearing the entire corolla lengthwise in two and then removing the halves, care being taken in so doing not to touch the stigma with any of the other parts of the flower. Most of the seed resulting from the back-cross was planted earlier than usual, in May and June of 1915; but the plants did not bloom before December — about the usual time of blooming. In all cases a record was made of the particular plant and capsule which produced the seed. Records were also made of the particular plant used as the pollen parent, and in certain cases also, of all the seeds resulting from the pollination with single anthers. The seed was planted in pans of sterilized soil, and the young seedlings transplanted to successively larger pots; about 800 plants were allowed to bloom in two and two-and-a-half inch pots, the rest matured in three, four and five inch pots. Individuals grown in small pots produced perfectly normal flowers (possibly a trifle smaller than those of plants in larger pots), but the number of flowers produced per plant was, of course, smaller.

An interesting feature in connection with the culture of primulas is the extreme cold in which they can thrive. The bulk of my plants was kept in cold-frames for the greater part of the winter, during which time they were probably subjected to temperatures considerably below freezing, since the outside temperature on several occasions was at or near zero Fahrenheit, and no artificial heat was used in the cold-frames.[5] These plants were much more vigorous and produced a better bloom than similar plants cultivated in the greenhouse.[6]

[5] Additional covering of boards or straw mats was applied at night.

It is also interesting that no watering during the entire winter was necessary, and although the soil became very dry, the plants did not droop or seem to suffer in any way. A few plants exposed by broken glass were killed by freezing.

[6] Commercial growers as a rule transfer primulas from cold-frames to the greenhouse late in fall, but I know of no previous instance in which they were kept outdoors all winter in a climate as cold as that of New York.

Primulas demand a light, rather dry soil in a moist, cool greenhouse (45°—60°F.). Over-watering is especially harmful to young plants before they become pot-bound; individuals so treated are sickly, yellow-leaved, with poorly developed flowers, the characters of which can not be well distinguished. Such plants must be discarded.

Whenever plants froze, failed to bloom properly, or were not included in the counts for any reason, a record of the fact was usually made.

A few further matters of technique should be mentioned. The factor for green stigma also makes the flower color, whether magenta or red, lighter. These light magentas and light reds are rather difficult to distinguish from each other at times. The flowers of poorly developed (especially over-watered) plants with green stigmas may appear light magenta, but the plant may really possess only the red factor. Any weak plants, therefore, with green stigmas and poorly developed flowers could not be included in the records, and were discarded. This would lower somewhat the number of individuals in classes 2, 3, 5, and possibly 8 (table 1 a), but as the number of such discarded individuals was very small (not over 25), these figures would not be appreciably altered. The light red and light magenta flowers of plants grown in small pots were as a rule not much, or no more, difficult to distinguish than the flowers of plants grown in larger pots. Especial care had to be exercised in not including in class 8 individuals which did not belong there, since a relatively few mistakes could alter appreciably the ratio of this class to the total. As a rule, where there was a possibility of confusion the plant was re-examined at a later period when new flowers were produced, and the two observations compared; and the individual was not recorded until there was certainty as to its proper classification. When there was any doubt as to the classification of the light individuals belonging to the larger classes 3 and 5, such plants were either re-examined later, or placed without re-examination in the class where they presumably belonged; in the latter case a note was always made of the extent to which the classification was questionable. Thus the color of twenty individuals of class 3 and of four of class 5 was somewhat doubtful. The color of fourteen individuals of class 3 and seven of class 5 was doubtful. There may have been several plants which belonged to class 8 but which were included in class 2, because all of the flowers on these plants appeared light magenta at the time the record was made, although the plants were light red genetically. This may explain partly the fact that class 8 is smaller than class 7, which it should

about equal. Moreover, a lot of about 300 plants grown in small pots is included in the records, and plants of this lot which were of uncertain color, especially such as might belong to class 8, were not classified. This again would lower somewhat the number of individuals of class 8.

The red stigma is most easily distinguished in the very young unopened flower. As the flower becomes older the stigma color becomes less distinct.

One hundred and eight plants grown in the cold-frames were killed by frost. There should be no resultant disproportionate lowering of any particular class or classes, since probably the frost had no selective action on one or more classes of plants. There were about 225 plants in small pots which I failed to count either because they had not produced a sufficiently abundant bloom at the time that I had opportunity to make counts, or because they were past their blooming period. These plants will be transferred to larger pots in an effort to induce them to bloom next winter.

The results of the back-cross are given in table 1. There are eight classes corresponding to the eight types of gametes, each class including one member of each of the three pairs of allelomorphs involved. It is desirable to report the results in this manner, as is explained below.

An examination of table 1a shows that the percentage of crossovers between the allelomorphic pairs *r R* and *s S* is 31.5 percent (column 2) + 2.52 percent (column 4) = 34.02 percent. That between *l L* and *r R* is 9.1 percent (column 3) + 2.52 percent (column 4) = 11.62 percent. When the factors are placed in the order given in table 1 (*l, r, s,* or *L, R, S*), a single crossover between the pairs of allelomorphs *l L* and *r R,* or between the pairs *r R* and *s S* results in a separation of, or crossover between, *l* and *s* (or *L* and *S*), but two simultaneous crossovers, one between *l L* and *r R,* the other between *r R* and *s S,* do not result in a separation of *l* and *s* (or *L* and *S*). The percentage of such simultaneous crossovers, or double crossovers, is 2.52 (table 1 a, column 4). If the F_1 had been homozygous for *r* (or *R*), there is no reason to doubt that essentially similar processes involving linkage would have taken place, viz., the same number of double crossovers would have taken place between the pairs of factors *l L, r r* (or *R R*), and *s S*; but the parental combinations or non-crossovers *l, r* (or *R*), *s,* and *L, r* (or *R*), *S,* would have been indistinguishable from the double crossovers *l, r* (or *R*), *s,* and *L, r* (or *R*), *S*. In other words, in an individual homozygous for *r* (or *R*), but heterozygous for *l L* and *s S,* we should be unaware of the existence of double crossovers, since the factors *l* and *s* (or *L* and *S*)

TABLE 1

a. *Offspring of back-cross* $\left(\frac{l, r, s}{l, r, s} \times \frac{l, r, s}{L, R, S}\right)$ *and reciprocal.*

Total number of individuals 3684.

l = long style r = red flower s = red stigma
L = short style L = magenta flower S = green stigma

	Column 1 Non-crossovers		Column 2 Single crossovers		Column 3 Single crossovers		Column 4 Double crossovers	
	1. *l, r, s*	1032	3. *l, r, S*	526	5. *l, R, S*	180	7. *l, R, s*	54
	2. *L, R, S*	1063	4. *L, R, s*	634	6. *L, r, s*	156	8. *L, r, S*	39
Sum of contrary classes		2095		1160		336		93
Percent of total (3684)		56.9		31.5		09.1		02.52

b. *Offspring of* $\left(\frac{l, r, s}{l, r, s} \times \frac{l, r, s}{L, R, S}\right)$ *only* (♂ *heterozygous*).

(Included above under a.)

	1. *l, r, s*	895	3. *l, r, S*	484	5. *l, R, S*	173	7. *l, R, s*	52
	2. *L, R, S*	934	4. *L, R, s*	569	6. *L, r, s*	152	8. *L, r, S*	33
Sum of contrary classes		1829		1053		325		85
Percent of total (3292)		55.5		32.0		0.9		2.55

c. *Offspring of* $\left(\frac{l, r, s}{L, R, S} \times \frac{l, r, s}{l, r, s}\right)$ *only* (♀ *heterozygous*).

(Included above under a.)

	1. *l, r, s*	137	3. *l, r, S*	42	5. *l, R, S*	7	7. *l, R, s*	2
	2. *L, R, S*	129	4. *L, R, s*	65	6. *L, r, s*	4	8. *L, r, S*	6
Sum of contrary classes		266		107		11		8
Percent of total (392)		67.6		27.2		2.8		2.21

would not separate and gametes containing them would as a result be indistinguishable from the non-crossovers. Any difference which exists between the sum of the number of single crossovers between *l L* and *r R*, and *r R* and *s S* on the one hand, and *l L* and *s S* on the other, is due to the double crossovers. These facts are directly indicated when the three factors, one from each of the three pairs of allelomorphs involved, are reported together; but if the F_1 is heterozygous for only two pairs of factors at a time (i.e., *l L* and *r R*, or *r R* and *s S*, or *l L* and *s S*), then any difference in the sum of the percentages of crossovers between *l L* and *r R*, and *r R* and *s S* on the one hand, and the percentage of crossovers between *l L* and *s S* on the other, may not represent a difference due to double crossovers, but may be due to variations in the percentages of crossovers which take place in different individuals at different times.

Geneticists are not agreed upon the explanation of linkage. BATESON's view that the various gametic ratios are due to reduplication implies a segregation of these types in different parts of the plant. It would follow on this hypothesis in its original form that entire anthers or ovaries should contain one gametic type. To test the latter view it was only

TABLE 2

Offspring resulting from pollinations with single anthers of the heterozygous plants. The record of these individuals is included in table 1.

Number of capsule.	*l r s*	*L R S*	*l r S*	*L R s*	*l R S*	*L r s*	*l R s*	*L r S*
(1) 4.2 a 1-2	36	43	18	30	5	7	0	0
(2) 4.2 a 2-1	24	33	16	33	12	11	2	1
(3) 4.2 a 2-2	48	48	26	41	11	11	4	1
(4) 4.2 a 2-3	21	21	6	14	6	8	2	0
(5) 4.2 a 2-4	20	26	8	12	4	9	2	0
(6) 4.2 a 2-5	17	18	10	12	4	4	0	0
(7) 4.2 c 1-2	5	8	3	6	3	2	0	0
(8) 4.2 c 1-3	3	8	2	5	1	0	0	1
(9) 4.2 c 1-4	18	13	9	10	0	0	1	1

Triple recessive plants (long style, red flower, red stigma) were pollinated with these anthers. The number followed by a letter (as 4.2 a) is the label given to a particular heterozygous plant; the number following the letter designates a particular flower of this plant, and the last number designates a particular anther of this flower. Thus, anthers recorded on lines (2) to (6) (4.2 a 2-1 to 4.2 a 2-5) all came from the same flower. Each line includes all the individuals from pollinations with a single anther only.

TABLE 3

Offspring grown from seeds of single capsules of the heterozygous plants. The record of these individuals is included in table 1a.

Number of capsule.	*l* *r* *s*	*L* *R* *S*	*l* *r* *S*	*L* *R* *s*	*l* *R* *S*	*L* *r* *s*	*l* *R* *s*	*L* *r* *S*
173	4	9	3	4	1	0	0	1
174	4	4	0	3	1	0	0	0
175	5	4	1	0	0	0	0	0
176	6	6	2	4	1	0	0	0
177	8	6	4	3	0	0	0	0
178	7	2	3	1	0	0	0	0
180	6	5	1	1	0	0	0	0
181	4	7	0	3	0	0	0	0
182	7	8	0	5	0	0	1	0
183	5	3	1	2	0	0	0	1
184	8	3	2	4	0	0	0	0
190	4	4	0	2	0	0	0	0
191	4	5	3	1	0	0	0	0
199	5	3	2	2	0	0	0	0
200	2	5	1	1	0	1	0	0
204	5	3	4	0	0	0	0	0
205	6	5	0	0	2	1	0	0
206	8	3	0	3	1	1	0	2
207	5	3	0	4	0	0	0	0
212	6	6	1	1	0	0	0	0
216	6	3	1	6	0	0	0	0
218	2	2	0	5	1	1	0	0

Only capsules are recorded from which more than ten seeds were planted and grown to maturity.

necessary, therefore, to keep separate, seeds resulting from the single heterozygous anthers or ovaries. Table 2 gives the results obtained by using single anthers. In each instance all the expected classes are realized (except the very small class of double crossovers in some cases); and they occur in about their expected proportions. The ovaries, as shown by table 3, gave similar results, but the number of seeds that are produced by a single capsule is too small (20—40) to make the ratios significant. Suffice it to state that in no instance (22 cases in all) did any heterozygous ovary produce only one type of gamete. It follows, therefore, that reduction must take place within the anthers and ovaries, owing to some peculiarity which must occur in cell processes and cell divisions at this stage. Such peculiarities are indeed found in the sporangia, namely, at the reduction divisions. This leads us to the other explanation of linkage, based on the parallelism in the distribution of

the chromosomes and of Mendelian factors. Especially striking and complete in all its details is the identical distribution of sex-linked factors and the sex chromosomes of Drosophila (MORGAN 1911). Thus, for instance, the cytological evidence shows that a male transmits his sex chromosome to his daughters only, not to his sons; he also transmits a certain group of factors (sex-linked) to his daughters only, and not to his sons. The results of all crosses show that wherever the sex chromosome goes, there also these factors go. Even when, in exceptional cases, the chromosomal distribution is abnormal, there is a corresponding abnormal distribution of these sex-linked factors (BRIDGES 1916). Because of this complete association, these factors are supposed to constitute, in part, the sex chromosome. Now, all sex-linked factors have been found to be linked to each other (a linkage group). All factors in the same chromosome, in other words, are linked. The chromosome contains many distinct (i.e., separable) factors, which are considered as occupying definite loci within it, in linear arrangement (STURTEVANT 1913, 1915).

This material continuity is broken only when there is an interchange of similar portions of homologous chromosomes resulting in a new combination of factors, or a crossover.[7] Factors that are allelomorphic are thought to occupy corresponding loci in the homologous chromosomes.

In harmony with all this observation and theory is the further fact that many other Mendelian factors which have been identified in Drosophila and which do not follow the sex chromosomes in their distribution to the offspring, are also never linked to those in the sex chromosome group. These non-sex-linked factors, however, form linkage groups of their own, and as the theory demands, these groups are equal in number to the pairs of chromosomes; and curiously enough, the number of identified factors in each group has so far been found to be roughly proportional to the size of the chromosomes (MULLER 1914, 1916). Finally, just as the pairs of chromosomes segregate independently of each other, so also any pair of factors in one linkage group segregates independently of any pair in all other linkage groups.

The experimental evidence that the segregation of sex-linked factors happens at the same time as the reduction of the sex chromosomes is complete. Here we are not dealing with theory, but with fact. The

[7] This term has been substituted for recombination by MORGAN; it carries a cytological implication besides referring to the observed breeding results.

inference as to similarity in the mechanism involved extends to those cases in which the homologous chromosomes are not differentiated. In these cases, the direct evidence could not be obtained, because one chromosome can not be distinguished from the other when in association, in an individual, with a Mendelian character.

In general, the facts of linkage find a rather simple explanation on the chromosome hypothesis. When only three linked factors are involved in an experiment, it is difficult or impossible to draw conclusions, from the mathematical relationships alone, of the probable nature of the processes which are responsible for these values. Such conclusions must be based on the values afforded by many factors, all linked, in which case the various complex or special mathematical relationships may be conceived of as the result of some spatial relationship of the factors, as is believed to be true on the hypothesis of linear arrangement of the factors in the chromosomes. It would therefore be desirable to get accurate data on a reasonably large series of linked factors in Primula or some other easily handled plant, for the sake of comparison with the results which have been gained on Drosophila and which have received, in general, a simple explanation on the chromosome hypothesis.

When an individual is heterozygous for three pairs of linked factors there are various proportions of the different classes of gametes possible on *a priori* grounds. I have attempted to establish accurately the particular values given in the case of Primula, especially the numerical proportions of the double crossover classes recorded in column 4, table 1a.

On the simple assumption that crossovers between *l L* and *r R* do not influence those between *r R* and *s S*, the percentage of crossovers between *l L* and *r R* (11.62 percent) should be as great when crossovers take place between *r R* and *s S* (i.e., 11.62 percent of 34.02 percent = 3.95 percent) as when the latter do not occur. From table 1, column 4, it will be seen that the percentage of double crossovers follows this simple assumption of their random occurrence, the realized percentage of 2.52 percent being somewhat below the expectation of 3.95 percent. The deviation from this value may be due to the fact that class 8 (column 4, table 1a) is lower than it really should be, as was explained above. The darker-colored classes 1, 4, 6 and 7, give more reliable results. If these classes alone are considered the double crossovers form 2.87 percent of the total (i.e., 54 = 2.87 percent of 1876). The percentage expected on the simple assumption of random occurrence of double crossovers is 2.80 percent (8.3 percent of 33.7 percent), a very close ap-

proximation to the realized percentage. These results would be explained on the chromosome hypothesis on the assumption that the factors have a linear arrangement in the chromosome in the order *l, r, s* (or *L, R, S*), and that there is no interference of crossing over between *l, r* and *r, s* (or *L, R,* and *R, S*). A double crossover, when the factors have this arrangement, does not result in the separation of the two outer factors (*l* and *s,* or *L* and *S*). The sum of the percentages of crossing over between *l* and *r,* and *r* and *s* is 45.64 percent (34.02 percent + 11.62 percent). The percentage of single crossing over between *l L* and *s S* is 31.5 percent + 9.1 percent = 40.6 percent. The difference between 45.64 percent and 40.6 percent is due to the double crossovers, for if the factors have a linear arrangement in the order mentioned, *l, r, s* (or *L, R, S*) a double crossover between *l* and *s* (or *L* and *S*) does not result in the separation of these factors.

This random occurrence of double crossovers occurs in Drosophila in cases involving rather loose linkage (i.e., a rather high percentage of crossovers). In cases involving tight linkage in this animal the number of double crossovers is below expectation. MULLER (1916) has pointed out this disparity in the latter case, and proposed the term "interference" for it. In our present state of knowledge, it is impossible to state whether one of the crossovers taking place tends to prevent the other, or whether they mutually prevent each other. In the latter case, there would be fewer total single crossovers than in the former case, other things being equal. We do not know, however, exactly what these percentages would be without interference, and therefore cannot tell how the latter operates.

On the other extreme, we are confronted with those cases in Drosophila in which very loose linkage is involved, and in which the number of double crossovers is far in excess of the simple expectation that one crossover does not involve the other. Between these two extremes of almost complete interference and of great excess of double crossovers there occur all intermediate values. These values are probably some function of the linkage strengths involved. Since, therefore, in different cases these values may be widely different, including all the mathematical possibilities, it is impossible to postulate with certainty any spatial relationship simply from the values given by only three pairs of linked factors.

Certain difficulties remain unsolved on the chromosome hypothesis. Cytology has not shown us clearly what the mechanism of crossing over

is, much less does it show us the cause of interference, although it suggests a number of possibilities. The loops of JANSSEN's (1905, 1909) chiasmatype seem too frequent for each to represent a crossover, and they are too short to account for the facts of interference. In this connection also we must consider some of the recent researches of BONNEVIE (1908) and of VEJDOVSKY (1911-12). The latter holds that the persistent element of the chromosome is really a long, thin, spirally wound thread or chromonema, lying within an envelope ordinarily considered the chromosome. If the factors have a linear arrangement, it must be along this chromonema. The best opportunity for the exact side-by-side juxtaposition of allelomorphic factors such as is demanded on the chiasmatype hypothesis would be at a stage when the chromosomes appear as long, thin threads, as in the leptotene spireme. At this stage the twists are extremely short and numerous and could hardly represent each a crossover; how one crossover would interfere with another at even short distances from it at such a stage is difficult to conceive, for such short distances themselves (say one-tenth of the length of the leptotene thread) contain numerous twists.

Finally, the different linkage values given by the two sexes in animals is an enigma. In Drosophila and moths no recombinations occur between linked factors in the heterozygous sex (male in Drosophila, female in moths). Although the details of synapsis have not been worked out in these forms, there seems to be no reason to doubt that they are essentially similar to those in Batrachoceps, in which the twisting of chromatin threads about each other has been observed in the heterozygous sex (the male); but it may be that crossing over occurs in the male Batrachoceps, as it does in the male mammal (rats, CASTLE and WRIGHT 1915).

So far as my figures go, no great dissimilarity in the linkage values given by the two sexes occurs in Primula. Table 1, b and c, gives these values for both the anthers and ovaries. The difference shown in the ratios is probably not significant, since the percentages of the various classes produced by the heterozygous females are based on small counts.

Some of my plants were grown at Columbia University, but most of them at the New York Botanical Garden.

SUMMARY

(1) In *Primula Sinensis* the pairs of factors studied may be arranged in the order: long or short style, red or magenta flower, red stigma or green stigma.

(2) Crossovers between the pairs of linked factors, long or short style, and red or magenta flower, respectively, take place without reference to the crossovers between the pairs of linked factors, red or magenta flower color and red stigma or green stigma, respectively; that is, there is no interference.

(3) Single anthers and single ovaries of the heterozygous individuals produce all classes of gametes. Reduction of factors must therefore accompany cell divisions occurring within many or all the anthers and ovaries.

(4) The proportions of the various gametic classes of linked factors is about the same for the anthers and the ovaries.

BIBLIOGRAPHY

BATESON, W., and PUNNETT, R. C., 1911 On gametic series involving reduplication of certain terms. Jour. Gen. **1**: 293-302.

BONNEVIE, K., 1908 Chromosomenstudien. Arch. f. Zellf. **1**: 450-514.

BRIDGES, C. B., 1914 The chromosome hypothesis of linkage applied to cases in sweet peas and Primula. Am. Nat. **48**: 524-534.

1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1**: 1-52, 107-163.

CASTLE, W. E., and WRIGHT, S., 1915 Two color mutations of rats which show partial coupling. Science N.S. **42**: 193-195.

GREGORY, R. P., 1911 a Experiments with *Primula Sinensis*. Jour. Gen. **1**: 73-132.

1911 b On gametic coupling and repulsion in *Primula Sinensis*. Proc. Roy. Soc. **84**: 12-15.

JANSSENS, F. A., 1905 Spermatogénèse dans les batraciens. III. Evolution des auxocytes mâles du *Batrachoceps attenuatus*. La Cellule **22**: 377-428.

1909 Spermatogénèse dans les batraciens. V. La theorie de la chiasmatypie. Nouvelle interprétation des cinèses de maturation. La Cellule **52**: 387-412.

MORGAN, T. H., 1911 An attempt to analyze the constitution of the chromosomes on the basis of sex-limited inheritance in Drosophila. Jour. Exp. Zool. **11**: 365-414.

MULLER, H. J., 1914 A gene for the fourth chromosome of Drosophila. Jour. Exp. Zool. **17**: 325-336.

1916 Mechanism of crossing-over. Am. Nat. **50**: 193-221, 284-305.

STURTEVANT, A. H., 1913 The linear arrangement of six sex-linked factors in Drosophila as shown by their mode of association. Jour. Exp. Zool. **14**: 43-59.

1915 The behavior of chromosomes as studied through linkage. Zeitschr. f. ind. Abst. u. Vererb. **13**: 234-287.

TANAKA, Y., 1914 Sexual dimorphism of gametic series in the reduplication. Trans. Sapporo Nat. Hist. Soc. **5**. 61-64.

1915 Occurrence of different systems of gametic reduplication in male and female hybrids. Zeitschr. f. ind. Abst. u. Vererb. **14**: 12-30

TROW, A. H., 1912 Forms of reduplication:—primary and secondary. Jour. Gen. **2**: 313-324.

VEJDOVSKY, W., 1911-12 Zum Problem der Vererbungsträger. 184 pp. Prag: Verlag der Königl. Böhm. Gesell. der Wissenschaften.

TRICOLOR INHERITANCE. II. THE BASSET HOUND[1]

HEMAN L. IBSEN

University of Wisconsin, Madison, Wisconsin

[Received June 6, 1916]

Part I of the present series deals with the inheritance of the tricolor condition in guinea-pigs. One of the reasons for undertaking that work was to shed some light on the inheritance of the tricolor coat in Basset hounds, which were studied biometrically by GALTON (1897) as a test of his "law of ancestral inheritance."

In the years intervening between the publication of GALTON's paper and the present it has been thoroughly established in a large number of mammals that color is inherited in Mendelian fashion. It seems perfectly reasonable to suppose, therefore, that the same is true in Basset hounds. The object of the present paper is to discuss the probable genetic factors involved in the production of the coat color in Basset hounds, their relation to each other, and test matings which could be made to determine how closely the factors and relationships proposed fit the actual cases.

CASTLE (1912) has given a rather brief and incomplete explanation on the Mendelian basis. It seems probable from some of his statements that he was not quite certain of the actual appearance of the Basset hound. A clear conception of the distribution of the color markings is necessary before one can hope to work out successfully the factors involved and their relationships. It therefore seems advisable, before proceeding farther, to sketch briefly the history and general appearance of the Basset hound, laying special stress on the distribution of the color markings.

The Basset is probably a very old breed of dog, having been known to exist in France for several centuries (BARTON 1910). It was first brought into prominence in England by Sir EVERETT MILLAIS, who imported from France in 1874 a famous Basset, called "Model."

In "The dog" (1881(?), p. 336), edited by VERO SHAW, appears a

[1] Papers from the Department of Experimental Breeding of the Wisconsin Agricultural Experiment Station, No. 8. Published with the approval of the Director.

letter by MILLAIS in which is given a description of the Basset. The letter is undated, but it is clear from the context that it was written in 1880, thus being one of the earliest if not the earliest English description of the breed. To quote from the letter, "Colour of course is a matter of fancy, although I infinitely prefer the 'tricolor,' which has a tan head and black-and-white body." In the same volume is a colored plate showing three prize-winning Bassets imported by GEORGE R. KREHL in 1881. All three have tan-and-white heads, with black patches on the back, but with the black running into tan on the hind quarters.

KREHL wrote a description of the breed for "The dogs of the British islands", edited by "STONEHENGE" (J. H. WALSH 1886). He gave what he called the "Points of the Basset hound." These were subsequently adopted by the Basset hound Club. Point 8 is, "The colour should be black, white and tan. The head, shoulders and quarters a rich tan, and black patches on the back. They are also sometimes hare-pied." Figure 1 represents what BARTON considers a "typical smooth-coated Basset bitch."

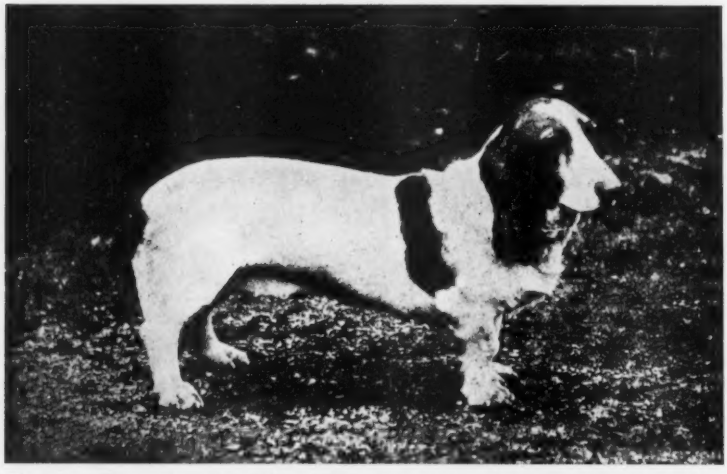


Figure 1. Basset hound. Characterized by BARTON as a "typical smooth-coated Basset bitch." Note the tan on the head and the small black patches on the back and at the base of the tail. (From BARTON 1910.)

It is evident from the above that the ideal to be striven for was quite early fixed in the minds of the breeders. In spite of this, however, it was found impossible to get the tricolors (black-white-and-tan) to breed

true. Tan-and-whites occasionally cropped out and these when bred together produced tricolors besides tan-and-whites. In a letter to the author, dated March 22, 1915, J. SIDNEY TURNER, editor of "The kennel encyclopedia," writes that tricolors "still throw a considerable number of tan-and-whites when bred together." It was this phenomenon which GALTON studied and to which he applied his law of ancestral inheritance.

Before going into a more detailed description of the color relations in the breed it is probably best to name and describe those factors pertaining to color which have already been studied in dogs and which may possibly have some application to color inheritance in Basset hounds.

LITTLE (1914) has made a study of coat color inheritance in Pointer dogs. He finds that two factors by their presence or absence determine all the colors produced. The factors are *B* and *E*. *B* is the factor for black, *b* for liver (chocolate or brown), *E* is the extension factor. When *E* is present it extends the black (*B*) or liver (*b*) so that the pigmented part of the coat is either black (*EB*) or liver (*Eb*). When *E* is absent and *B* present, i.e., *eB*, the black is not extended and is found only on the nose, the pigmented part of the coat being tan (red or yellow). When *B* also is absent, i.e., *eb*, the pigmented part of the coat is tan and the nose brown. LANG (1910) had previously determined that black is dominant to liver or brown.

BARROWS and PHILLIPS (1915) worked with Cocker spaniels. They find that the factors *B* and *E*, in addition to others controlling white spotting, dilution, etc., are concerned in the color inheritance of these dogs also. What is of special interest in connection with their work, because of its application to the Basset hound color, is their study of the "bicolor" condition. By "bicolors" they mean black-and-tans, liver-and-tans and "red[tan]-and-lemons." A black-and-tan Cocker spaniel is a "black dog having dark or light red or lemon spots over each eye, and extended red areas distributed on the sides of the muzzle, inside of the ear, posterior surfaces of the legs, and on the ventral sides of the chest, abdomen, and tail" (BARROWS and PHILLIPS 1915, p. 395). A liver-and-tan is liver(chocolate)-colored where a black-and-tan is black. In a red-and-lemon the body color is red, while those portions which are tan in a black-and-tan are here lemon in color. BARROWS and PHILLIPS apply the term "bicolor" only to the colors mentioned above and not to dogs showing a combination of one of these colors with white. The black-and-tan pattern as found in Cocker spaniels is probably the one usually found in dogs. Some black-and-tan breeds, as for instance Airedale terriers, have more than the usual amount of tan. According

to the Airedale "standard" the "head and ears, with the exception of dark markings on each side of the skull, should be tan, the ears being of a darker shade than the rest. The legs up to thighs and elbows being also tan. The body black or dark grizzle."

My own experience with self black, tortoise and self red guinea-pigs (IBSEN 1916) had led me to suppose that black-and-tan in dogs might correspond to tortoise in guinea-pigs. In Part I tortoise in guinea-pigs was interpreted as being due to a modification of the extension factor (E), being the middle term in the allelomorphic series E, e^t and e. Such, however, does not seem to be the case in dogs. BARROWS and PHILLIPS state that the bicolor condition is recessive to the non-bicolor or self-colored. Since both self black (E) and self red (e) dogs are non-bicolors and bicolor is recessive to both, it is evident that the bicolor condition is not due to a modification of the extension factor (E), but to some entirely independent factor.

Although BARROWS and PHILLIPS determined the general relation of bicolor to the self-colored condition, they made no attempt to treat the relation factorially. This I am doing by introducing the factor T. When T is present the animal is self-colored; in its absence it is bicolored. In the presence of E the bicolored condition manifests itself as black-and-tan; in the absence of E as red-and-lemon. In order that complete extension of black or chocolate pigment be realized it is therefore necessary that T be present as well as E. The relation of T to the other factors may be shown in the following manner:[2]

BET = self black,
BEt = black-and-tan,
BeT = self tan (red) with black nose,
Bet = red-and-lemon with black nose.

On this interpretation a self tan of the formula $BBeeTT$ mated to a black-and-tan ($BBEEtt$) should have only self black offspring ($BBEeTt$). There is no record of such a mating in BARROWS and PHILLIPS's paper.

The black-and-tan factor in dogs differs decidedly from the factor for a pattern with the same name in rabbits. In the latter it is a modification of the agouti factor and forms an allelomorphic series with agouti (A) and non-agouti (a) (CASTLE and FISH 1915). There are also black-and-tan mice but the inheritance of the pattern in these animals has not as yet been reported on.

[2] I am assuming for the sake of simplicity that the animals are entirely pigmented, i.e., there is no white.

There is one more color factor in dogs which should be mentioned. BARTON (1910), in the section on Dachshunds, writes,

"As a rule, the crossing of a black-and-tan Dachs with a red one, produces puppies true to type, i.e., some are black-and-tan, others red, and not a mixture of these two colours. *A red dog and bitch, will, however, sometimes throw a black-and-tan puppy*,[3] or a black-and-tan sire and dam produce a whole-red puppy. This is precisely what happens with certain other varieties."

It seems, therefore, that in dogs we have a red which is dominant to black-and-tan. HAGEDOORN (1912) also makes mention of this factor. The probabilities are that it is a restriction factor which inhibits the production of black pigment in the coat. I am calling this factor *R* (restriction or dominant red). If we can believe the statements of fanciers the *R* factor is found in rabbits also, since Rufus Reds bred together may have self black offspring.

We are now in a position to take up somewhat in detail the inheritance of color in the Basset hound. It is evident that the tricolor coat is due to black-and-tan plus white spotting. The tan covers more of the body than it does in most black-and-tans, but there are breeds, such as the Airedale terriers, previously mentioned, which have as much tan and as comparatively little black as have the Bassets. The white spotting may be disregarded factorially because it is always present. Besides, its exact relation to the entirely pigmented coat has not as yet been satisfactorily settled, and it would only unnecessarily complicate matters to bring in the discussion of this relation.

Tricolor Bassets never have liver-colored (chocolate or brown) spots instead of black, and they must therefore be homozygous for black (*B*). They may, however, have progeny without any black spotting whatever, which makes it fairly certain that they can be heterozygous as well as homozygous for *E*. This will be discussed later. They must always be homozygous for the absence of *T*, because if *T* were present none of the tan would show and we would have black-and-whites instead of tricolors. There is no record of any black-and-whites ever occurring among Basset hounds. The *R* factor must also be absent, otherwise the dog would be a tan-and-white instead of a tricolor. The zygotic composition of a tricolor Basset hound may therefore be put down (disregarding white spotting) as either *BBEEttrr* or *BBEettrr* depending on whether it is homozygous or heterozygous for *E*.

At this point it will be necessary to consider the distribution of white

[3] Original not italicized.

spotting in dogs in order to show how it may affect the appearance of the black-and-white pattern. ALLEN (1914) has given diagrams (figure 2) showing the usual location of the white areas in a series of dogs

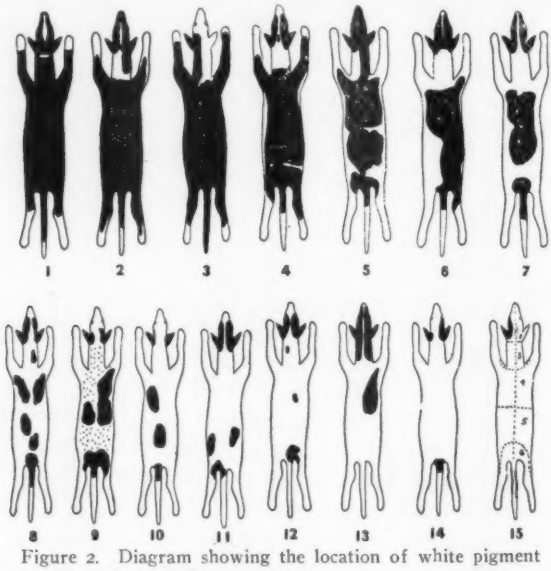


Figure 2. Diagram showing the location of white pigment in a series of dogs (ALLEN 1914).

from those which are largely pigmented to those which have only a small amount of pigmentation. ALLEN used these diagrams to illustrate the fact that as the pigment decreases in amount it draws towards definite centers from which it disappears last. He did not point out what also appears to be a fact in dogs, namely, that the last centers from which the pigment disappears are the ones designated by him the aural patches. It will be noted that every dog in his diagrams has pigment on the head even though the rest of the body is entirely white. It is significant for our purposes that if a dog has the pigment reduced to a single area this is always located on the head, so far as observation goes. This condition is not unusual in some breeds, such as fox-terriers, and may be seen occasionally in a number of others.

We may now take up in some detail the possible kinds of offspring resulting from the mating together of tricolors. The first type of mating to be considered will be that in which at least one parent is homozygous

for the extension factor (E), thus insuring that all the offspring shall carry the E factor. This means in Basset hounds that the head, shoulders and quarters are tan with black patches on the back, providing there is a fairly small amount of white spotting. TURNER, in the previously quoted letter, states, "I remember that MILLAIS told me that it was necessary to return to heavily marked blacks to keep up the tricolours." This means that the offspring of tricolor Bassets oftentimes have a larger amount of white than the parents. If figure 1 is, as BARTON states, a typical Basset, it can easily be seen how a comparatively slight reduction in the amount of pigmentation would leave only the head pigmented. Animals pigmented in this manner would be tan-and-whites. If tricolor Bassets had the more usual black-and-tan pattern (see p. 369), which means the presence of black areas on the head also, the number of E tan-and-whites would be greatly decreased, since it would require more white to blot out all the black. In order for a black-and-white Basset to be obtained from tricolor parents it would be necessary that the entire head, shoulders and quarters be white and that there be a pigmented patch (black) on the back. This, as we have seen, never occurs in dogs. Black-and-whites may be obtained from tricolor guinea-pigs because of the fact that there is no regularity in the distribution of the black and red, and also because there is no regularity in the distribution of the white areas.

Tan-and-white Bassets of tricolor parentage carrying E are necessarily, therefore, pigmented only on the head, and when bred together should get tricolor offspring as well as tan-and-white. I have never seen any photographs of tan-and-white Bassets, but in answer to a query, TURNER, in a letter of March 22, 1915, replies, "Tan is often found on the back of tan-and-white (no black) Bassets and foxhounds." This implies that those with tan only on the head are to be found, and the probabilities are that many of them carry E. In the next paragraph tan-and-whites with tan spots on the back as well as on the head will be discussed.

If Ee tricolors are bred together we may expect somewhat different results. There is always the possibility of getting E tan-and-whites, pigmented only on the head, but in addition we would expect one-fourth of the offspring to be tan-and-whites due to the absence of E. These may or may not have tan spots on the back, depending on the amount of white. We see from the foregoing that tan-and-whites pigmented only on the head, from tricolor parents, may or may not carry the E factor; but tan-and-whites pigmented also on the back, from tricolor

parents, are always *ee* and should therefore breed true. We should expect these animals to have a somewhat lighter shade to the tan spots on the head than to the tan spots on the back since they are of the formula *BBeettrr* and hence white-spotted red-and-lemons. It is impossible to determine from GALTON's data whether or not tan-and-whites ever breed true or whether they ever show the red-and-lemon pattern. In the studbooks the distribution of the tan spots is never denoted and it seems that the only method whereby these questions can be settled is by actual breeding tests.

So far we have avoided the *R* factor by starting out with parents both of which were tricolors and hence *rr*. There is no way at present of determining whether or not this factor is found in the Basset hound. If it were, all the animals carrying it would necessarily be tan-and-whites. It is possible that they might differ in some respects from the previously described tan-and-whites, but nothing seems to be known on this subject. One could be certain that the factor existed in the breed if two tan-and-whites with tan patches on the back should get tricolor offspring. This would indicate that both parents were *Rr*.

GALTON (1897) states that among the 818 animals whose colors were known to him there was one which was described as a black-and-tan.[4] CASTLE (1912) thinks this corresponds to the black-and-whites found in the tricolor series in guinea-pigs. HAGEDOORN (1912) on the other hand is of the opinion that the animal in question was a tricolor showing very little white and therefore bringing the black-and-tan pattern more into evidence. This explanation seems reasonable.

HAGEDOORN makes other statements which seem less incontrovertible. He states,

> "In those cases in which two tricolor parents gave lemon-and-white offspring, I feel sure, such young were of that color only because they happened not to be pigmented in a spot where sable [similar to black-and-tan] dogs show black color."

In other words tan-and-whites are genetically tricolors in which the areas that would normally be black are white, but in which some tan spots remain. This would assume that tricolors are always *EE* and therefore may get *E* tan-and-white but never *ee* tan-and-white offspring. He thinks, since tricolors are always black on the back, that all tan-and-whites with tan spots on the back must carry a dominant red factor (*R*). He fails to recognize that tan-and-whites of this description may result from the loss of the extension factor.

[4] This individual was disregarded by GALTON in his inheritance studies.

The question as to the presence of the factor *R* in Basset hounds, as well as some of the other unsettled points, can only be determined by more detailed records or by actual breeding tests.

SUMMARY

1. Basset hounds were imported into England from France by Sir EVERETT MILLAIS in 1874.

2. There are two kinds, (1) tricolors and (2) tan-and-whites. Black-and-whites do not occur in the breed. Tricolors are described as "black, white and tan. The head, shoulders and quarters, a rich tan, and black patches on the back." Tan-and-whites have tan heads and "tan is often found on the back."

3. The factors involved in color inheritance in Basset hounds are: (1) *B*, the factor for black, always present in Bassets. (2) *E*, the extension factor, which extends the black (or chocolate) and may be present or absent. (3) *T*, the factor for uniform pigmentation; animals without *T* are either black-and-tan, liver-and-tan or red(tan)-and-lemon. *T* is always absent in Bassets. (4) *R*, the factor which inhibits the formation of black (or chocolate) pigment in the coat. It is questionable whether this factor is ever present in Bassets.

4. By means of ALLEN's (1914) diagrams of distribution of pigmentation in dogs and by reference to various breeds, etc., it is demonstrated that pigmented dogs always have some pigmentation on the head; when pigmentation is very slight the head is often the only place where it is to be found. Bassets pigmented only on the head are tan-and-whites. Black-and-whites never occur because the head is never entirely devoid of pigment in this breed.

5. Tricolors may be of the formula *BBEEttrr* or *BBEettrr*. *Ee* tricolors mated together get some *ee* offspring which are tan-and-whites and which should breed true. These may have tan spots on the back. In this case, when *T* is absent, the tan on the head should be of a lighter shade than the tan on the back.

6. If *R* is present in Bassets then *BBEE*(or *Ee*)*ttRr* tan-and-whites (with tan on the back) bred together should get some tricolor offspring. This cannot be determined from available data.

LITERATURE CITED

ALLEN, G. M., 1914 Pattern development in mammals and birds. Amer. Nat. **48**: 385-412.

BARROWS, W. M., and PHILLIPS, J. McI., 1915 Color in Cocker spaniels. Jour. Heredity **6**: 387-397.

BARTON, F. T., 1910 Sporting dogs: their points and management. pp. xx + 450. New York: William R. Jenkins Co.

CASTLE, W. E., and FISH, H. D., 1915 The black-and-tan rabbit and the significance of multiple allelomorphs. Amer. Nat. **49**: 88-96.

GALTON, F., 1897 The average contribution of each several ancestor to the total heritage of the offspring. Proc. Roy. Soc. London **61**: 401-413.

HAGEDOORN, A. L., 1912 On tricolor coat in dogs and guinea-pigs. Amer. Nat. **46**: 682-683.

IBSEN, H. L., 1916 Tricolor inheritance. I. The tricolor series in guinea-pigs. Genetics **1**: 287-309.

LANG, A., 1910 Über alternative Vererbung bei Hünden. Zeitschr. f. ind. Abst. u. Vererb. **3**: 1-33.

LITTLE, C. C., 1914 Coat color in Pointer dogs. Jour. Heredity **5**: 244-248.

SHAW, V., 1881 (?) The dog. viii + 664 pp. London: Cassell & Co.

WALSH, J. H., 1886 The dogs of the British Islands. xvi + 292 pp. London: "The Field" Office.

TRICOLOR INHERITANCE. III. TORTOISESHELL CATS[1]

HEMAN L. IBSEN

University of Wisconsin, Madison, Wisconsin

[Received June 6, 1916]

DONCASTER has published several papers dealing with the inheritance of color in cats, the first of which appeared in 1904. LITTLE (1912) and WHITING (1915) have also written on the same subject. Both DONCASTER and LITTLE have established that the colors orange (yellow) and black are sex-linked. According to them the female is homozygous and the male heterozygous for sex. On their interpretation a female bearing the orange factor in one chromosome and the black factor in the other is a tortoiseshell, i.e., an animal spotted with black and orange. Black and orange are, accordingly, allelomorphs, but neither is dominant to the other. Orange females are represented by DONCASTER (1913) as *YX.YX;* orange males, *YX.bx;* black females, *BX.BX;* black males, *BX.bx;* and tortoiseshell females, *BX.YX*. LITTLE's method of representing the above color types is essentially the same as DONCASTER's.

For the normally expected color types DONCASTER and LITTLE have the same interpretation, but they differ as to the origin of the unexpected classes (see table 1). LITTLE considers the rare tortoiseshell male a mutation, while DONCASTER thinks it may be due to crossing over in the male. The latter has presented his idea in the following manner:

Black female, *BX.BX* × *YX.bx*, yellow male

Gametes, *BX*, *BX* *Yx*,*bX*

Black female, *bX.BX* *Yx.BX*, tortoiseshell male

The above scheme would also account for the unexpected class of black females which occurs in the black female × yellow male mating.

DONCASTER admits that there are some difficulties connected with his interpretation. Among these are his *bX.BX* black females, which as he says should give, when mated to orange males, not only tortoiseshell females but also *YX.bX* orange females. He fails to point out that

[1] Papers from the Department of Experimental Breeding of the Wisconsin Agricultural Experiment Station, No. 9. Published with the approval of the Director.

TABLE 1
DONCASTER'S *cat data.*

Parents	Offspring					
	Females			Males		
	Orange	Black	Tortoise-shell	Orange	Black	Tortoise-shell
1. Black ♀ × orange ♂		**13**	48		46	**1**
2. Orange ♀ × black ♂			16	20		
3. Tortoiseshell ♀ × orange ♂	47	**5**	43	54	38	**1**
4. Tortoiseshell ♀ × black ♂		12	21	35	29	**1**
5. Orange ♀ × orange ♂	40		**3**	48		

This table gives DONCASTER'S (1913) data. The offspring about which there was any doubt are not included. The numbers in heavy type represent the individuals of the normally unexpected classes.

they should in addition give *bX.bx* males. Since *b* means the absence of both black (*B*) and orange (*Y*) one would have to assume that these males are neither orange, black nor tortoiseshell. He also states that tortoiseshell or black females mated to tortoiseshell males do not get tortoiseshell male offspring as one would expect by his hypothesis. Another criticism of much greater weight than any of the foregoing is that he has crossing over of a sex-linked factor taking place in the male, which is assumed to be heterozygous for sex. This is contrary to all known facts, for in those cases even in which a *Y* chromosome is known to be present it has never been demonstrated that it carries any hereditary factors.[2]

WHITING'S (1915) explanation for the unexpected color types is entirely different from DONCASTER'S. He thinks that the black females in matings No. 1 and 3 (table 1) are in reality tortoiseshells which have had the black pigmentation extended to such a degree that little or no orange is visible. It is quite possible that some of the unexpected blacks were obtained in this manner. I have obtained a few "self" black guinea-pigs from tortoise × tortoise matings (IBSEN 1916), but it seems improbable that over 20 percent of the total female offspring in mating No. 1 should be accidental blacks, as would be necessarily the case on this hypothesis.

[2] JOHANNSEN (1913, pp. 609-612) believes DONCASTER is not justified in postulating sex chromosomes as bearers of the factors for black and orange. He therefore makes some modifications of DONCASTER'S interpretation in order that the factorial treatment may be more "purely Mendelian." Male cats are represented factorially as *Mm* and females as *mm*, and the factors for black and orange are assumed to be rather closely linked to *m*. Even with these modifications the hypothesis is essentially the same as DONCASTER'S and is open to practically the same objections.

WHITING also discusses the possibility of white spotting affecting the phenotypic appearance of an animal. In a tortoiseshell the white areas may fall, he suggests, on those spots which would otherwise have been orange and thus leave only the black pigmentation visible. Such an animal would be a black-and-white in appearance. This condition is well known in guinea-pigs. Unless, however, the amount of white spotting is relatively large with respect to the amount of yellow the chance of its all being covered, so as to produce a black-and-white, is very small (IBSEN 1916). According to BARTON (1908) a piebald (black-and-white) is usually white only on the face, breast and feet. If any of the blacks in mating No. 1, therefore, had been white even to this extent, it is probable that they would have been classified as piebalds rather than as blacks. Even though they were not so classified, it is still improbable that the usual small amount of white would have covered all of the yellow.

The rare tortoiseshell male, WHITING suggests, is "genetically a yellow with an extreme of black extension factors or a black with an extreme of yellow extension factors." This hypothesis lacks definiteness, and is practically impossible to prove or disprove.

From what has preceded it is evident that the hypotheses offered are far from perfect in that they either fail to explain adequately all the facts or are difficult to test experimentally.

I have been attracted to the problem of inheritance in the tortoiseshell cat because of its resemblance to the tortoise guinea-pigs with which I have been working. In guinea-pigs the self black condition is dominant to tortoise, but it is evident that this is not the case in cats. Tortoise in guinea-pigs is due to a definite single factor, while in cats DONCASTER and LITTLE assume it is due to the interaction of the black and orange factors. WHITING postulates extension factors governing the relative amounts of black and orange.

It seems possible to explain many of the apparent anomalies of color inheritance in cats by assuming that the tortoiseshell coat is due to one definite factor, which I have called *T*, and which can act only in the presence of black (*B*), causing the black to be restricted to spots and leaving orange areas between. Two other assumptions are necessary in order to explain all the facts; first that black (*B*) is dominant to orange (*b*), as in guinea-pigs, rats, dogs, etc., and second, that under ordinary conditions *T* (tortoiseshell) is closely linked to *b* (orange). These points can be brought more clearly to mind by an inspection of figure 1,

in which the factors are represented as being lineally placed on the sex chromosome. It will be noted that two sets of allelomorphs are assumed rather than one as was postulated by DONCASTER and LITTLE. It must also be kept in mind that since *T* acts only in the presence of *B* (black) it will have no visible effect on males carrying the *b* (orange) factor or on females homozygous for this factor.

So long as *T* and *b* remain on the same chromosome the hypothesis here presented is as efficient as DONCASTER'S or LITTLE'S in explaining the normally expected classes in the different matings. It is by means of the occasional crossing over of the factors that the attempt will be made to explain the unexpected classes.

At the outset it may be stated that the hypothesis accounts for the unexpected black females and all the tortoiseshell males, except the one

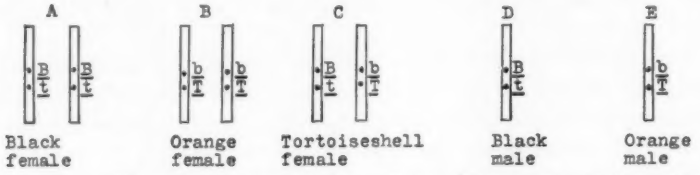


Figure 1. Diagrammatic representation of sex chromosomes bearing color factors showing normal condition in which *T* and *b* (orange) are postulated to be closely linked.

in mating No. 1, which, however, may be explained by a combination of WHITING'S hypothesis and my own. The tortoiseshell females in mating No. 5 are explained on WHITING'S hypothesis, which seems adequate in this particular case, as will be explained later.

I shall first take up the way in which tortoiseshell males may be produced. Since *T* and *b* are by hypothesis closely linked, we must assume that crossing over takes place only rarely, and it can occur only in the female. Its occurrence in a homozygous female (figure 1, A or B) would produce no effect in the offspring. The heterozygous female (figure 1, C) is a tortoiseshell and normally forms gametes *Bt* and *bT*. When crossing over takes place gametes *BT* and *bt* are produced. If this female were mated to an orange male, *bT*.—, the following classes would occur as a result of the crossing over:

BT.bT, tortoiseshell ♀,
bt.bT, orange ♀,
BT.—, *tortoiseshell* ♂,
bt.—, orange ♂.

This would account for the tortoiseshell male in mating No. 3. With a black male, *Bt.*—(figure 1, D), the offspring resulting from the crossing over are,

BT.Bt, tortoiseshell ♀,
bt.Bt, black ♀,
BT.—, tortoiseshell ♂,
bt.—, orange ♂,

thus accounting for the tortoiseshell male in mating No. 4. DONCASTER (1913) states that he knew of several tortoiseshell males besides those listed in table 1, all of which came from tortoiseshell females by unknown sires. It would therefore seem that tortoiseshell males, when they occur, come almost invariably from tortoiseshell females. This agrees with my hypothesis, according to which tortoiseshell males can be produced only by tortoiseshell mothers. The one recorded in mating No. 1 as having a black mother is an apparent exception. I have no explanation for this case, unless perhaps as already intimated the female was in reality a tortoiseshell, but with so little orange showing that she was recorded as a black.

In the paragraphs just preceding I have tried to indicate how tortoiseshell males are produced. I shall next show what kind of offspring are expected from them.

DONCASTER records the mating of a tortoiseshell male with a black female. The female was not kept in confinement after copulation was observed, and DONCASTER says there may be some doubt as to the paternity of the offspring. The only offspring recorded are a black male and tortoiseshell female, which are exactly what would be expected by hypothesis:

Black ♀,	*Bt*	*Bt*
Tortoiseshell ♂,	*BT*	—
F_1	*Bt.BT,* tortoiseshell ♀	
	Bt.—, black ♂	

When tortoiseshell females are mated to tortoiseshell males DONCASTER states that the following four types of offspring are obtained: tortoiseshell females, *orange females,* orange males, and black males. On my hypothesis no orange females should result.

Tortoiseshell ♀, *Bt* *bT*
Tortoiseshell ♂, *BT* —

F_1 { *Bt.BT*, tortoiseshell ♀
bT.BT, tortoiseshell ♀
Bt.—, black ♂
bT.—, orange ♂

It will be noted, however, that the second type of tortoiseshell female in the F_1 generation has a double dose of *T* and a single dose of *B*. It may be possible that the extra *T* restricts the black to such an extent that the animal appears to be an orange instead of a tortoiseshell. This is offered only as a suggestion.

A further test of the hypothesis would be to determine what kind of offspring are obtained from the F_1 females resulting from the tortoiseshell × tortoiseshell cross. By hypothesis one-half of their male offspring should be tortoiseshells no matter what the color of the male is to which they are mated. There appears to be no record of a mating of this type.

We may next consider how the black females in matings No. 1 and 3 may have been formed. It will be remembered, that when crossing over takes place in the tortoiseshell female, the two kinds of crossover gametes formed are *BT* and *bt*, and that no matter whether the male parent is black or orange the male offspring are *BT.*—, tortoiseshell, and *bt.*—, orange. The *bt.*— orange male is different from other orange males in that *b* is not linked with the *T* factor. By the mating of this sort of orange male with (1) a black or (2) a tortoiseshell female we should obtain black females which are otherwise unexpected in matings No. 1 and 3.

(1) Black ♀, *Bt* *Bt*
Orange ♂, *bt* —

F_1 { *Bt.bt*, *black* ♀
Bt.—, black ♂

(2) Tortoiseshell ♀, *Bt* *bT*
Orange ♂, *bt* —

F_1 { *Bt.bt*, *black* ♀
bT.bt, orange ♀
Bt.—, black ♂
bT.—, orange ♂

It is to be regretted that DONCASTER does not give the offspring from individual matings. If this had been done it would have been possible to test more thoroughly that part of the hypothesis dealing with the unexpected black females. It will be noted that when black females are mated to *bt.*— orange males all the offspring should be black. There is no way of determining from DONCASTER's data whether or not this ever happens. Moreover, when tortoiseshell females are mated to *bt.*— orange males none of the female offspring should be tortoiseshells.

Part of DONCASTER's data were obtained from BONHOTE. These are now given separately in BONHOTE's (1915) recent book. Here the individual matings are presented and it is possible to trace the offspring of three orange males. Two of the males had the usual tortoiseshell and orange daughters when mated to either black or tortoiseshell females, while the third had 1 orange, 3 *black*, 6 *blue* and 7 tortoiseshell female offspring when similarly mated. This last mating does not fit in at all with the theory that the orange male parent was *bt.*—. It should be pointed out, however, that, as BONHOTE states, he always selected tortoiseshell mothers carrying a large amount of black, which would facilitate the production of tortoiseshell daughters with large amounts of black also. Some of these daughters might therefore have the appearance of blacks. It will also be noted that many (6) of the blacks were dilute and hence classified as blues. I have noticed in dilute tortoise guinea-pigs that it is much more difficult to detect the small yellow (dilute red or orange) spots surrounded by dilute black hair, than it is to detect the small red spots surrounded by deep black hair. It is therefore possible that in this way also animals which should have been classified as tortoises have been called blacks (blues).

The crossover (*bt.*—) orange male should be as rare as the tortoiseshell male. The latter is often sterile and it is possible the *bt.*— orange male is sometimes sterile also. Taking all this into consideration it is probable that matings between either black or tortoiseshell females and *bt.*— orange males are comparatively infrequent; thus it is quite possible that DONCASTER has no record of this type of mating.

If such matings occur, however, the black female offspring would be of the formula *Bt.bt.* These bred to either orange or black males should have orange and black sons in equal proportions. The orange sons would be *bt.*—. On WHITING's hypothesis the unexpected black females should have orange and black sons also, but the orange sons should be *bT.*—. DONCASTER states that he knows of "no satisfactory record of a yellow male mated to a black female having yellow sons." From this

one may infer that there have been cases reported in which a black female had orange sons, but none of them so far have been thought reliable. A larger number of records may furnish some reliable cases.

I have tried so far to account for the tortoiseshell males and the unexpected black males. The three tortoiseshell females in mating No. 5 remain to be explained.

BARTON (1908), writing for the fancier, states, "If there is no white [in a tortoiseshell], then the amount of black hair should be small, compared with the red [orange] and yellow [dilute orange] markings." According to DONCASTER the three tortoiseshell females in mating No. 5 come from one mating. It is therefore possible that in selecting for a tortoiseshell female with the requisite small amount of black spotting one was obtained showing so little black that it appeared to be an orange. Mated to a true orange male this should produce some tortoiseshell female offspring. WHITING has given essentially the same explanation in his 1915 paper.

While it must be admitted that the foregoing hypothesis is not entirely satisfactory, and carefully controlled experiments are necessary for its substantiation, it still has two decided advantages, (1) it is quite definite, thereby tending to be comparatively easy to prove or disprove, and (2) it violates none of the accepted tenets of genetics.

GENERAL COMPARISON OF TRICOLOR IN GUINEA-PIGS, BASSET HOUNDS AND CATS

In parts I, II and III of the present series characteristics of the tricolor coats of guinea-pigs, Basset hounds and tortoiseshell cats have been considered separately with little reference to their resemblances or differences. They will now be considered briefly from this general comparative viewpoint. We shall first take up the spotting characters themselves, after which their factorial relations will be discussed.

The tricolor coats of guinea-pigs, and tortoiseshell cats show a much greater resemblance to each other than do either of them to the tricolor coat of Basset hounds. They will therefore first be compared with each other and then both can be compared with the Basset hound coat.

Since white spotting tends to mask the true black-red relationship, it is better first to consider black and red alone in their relation to each other, and then to take up white spotting as it affects both of them. In both cats and guinea-pigs the black spotting is variable in amount and is quite irregularly distributed. In both, black may be so far extended that

the animal looks like a self black or so little extended that it resembles a self red. Where the animals differ from each other is in the white spotting. In tortoiseshell cats, as bred by the fanciers, this is small in amount and has therefore little chance of blotting out all of the orange (red) or all of the black. In guinea-pigs, on the other hand white varies greatly in amount and distribution. It can therefore at times blot out either the black or the red and in this way produce red-and-whites or black-and-whites instead of the usual tricolors. Thus we see in guinea-pigs that the variability in amount and distribution of both the black and the white spotting help to produce the unusual color types, while in cats this is affected only by the black spotting. Because of this, cats tend to have fewer of the unusual types than do guinea-pigs.

Basset hounds differ decidedly in several respects from both tortoiseshell cats and tricolor guinea-pigs in that black is localized on the back, so that if there were no white present the entire head, legs and belly would presumably be tan in color, making the animal a black-and-tan. There is no chance here for the black to be so far extended that the animal appears to be a self black or so little extended that it appears to be a self red. The distribution of the white is also somewhat different. In both guinea-pigs and dogs, as well as in mammals in general, pigmentation tends to recede toward definite centers, and each of these centers may become entirely devoid of pigmentation. The order in which the centers become pigmentless seems to be quite irregular in guinea-pigs, while in dogs there is greater regularity. The point that concerns us in the present instance is that the aural patches are the last from which pigmentation entirely recedes, and as already noted, these patches in the Basset are always tan. As a consequence the dog may be entirely white except for these tan patches covering the ears. Such an animal is a tan-and-white. Black-and-whites never occur since black pigmentation is never found on the head in Bassets.

Summarizing, we find that in cats white spotting plays a very unimportant rôle in the production of red-and-whites and black-and-whites; black spotting is perhaps occasionally responsible for these color types. In guinea-pigs black spotting and white spotting are co-equal in their effects, while in Basset hounds white spotting is chiefly instrumental in the production of tan-and-whites, the black spotting merely being passive and aiding only by its position.

So far we have been considering in the three species only the visible relations of black and of white spotting to red. We may next take up

their factorial bases. The white spotting factors in the three animals may have some resemblances so far as we know, but since white spotting has not as yet been adequately treated in a factorial manner, we are not in a position to discuss them. The black spotting factor, on the other hand, can be more definitely discussed.

In guinea-pigs this is called the partial-extension factor (e^p) and is the middle term of a triple allelomorphic series of which entire extension (E) and non-extension (e) are the two extremes. It is therefore recessive to entire extension. In cats on the other hand the factor for black spotting is assumed to be a dominant partial-restricting factor (T), sex-linked, and also closely linked to the orange factor (b). Here it is dominant to entire extension of black. In Basset hounds two factors have to be considered. E is present just as in guinea-pigs, but black spotting here is not due to a modification of E as it is in guinea-pigs. Instead we have a new factor (T) which is not found in either guinea-pigs or cats. The T in cats and the T in dogs are two entirely different factors. As found in dogs it is the factor for the self-colored condition. In its absence (t) the animal is bi-colored. Since a bi-colored animal may be a red-and-lemon as well as a black-and-tan we see that t differs from e^p in guinea-pigs and T in cats in that it has to do with spotting with red as well as spotting with black or chocolate. In order to get dogs spotted with black but not with red, E and T must both be present.

We thus see that though characters in different animals may show some resemblances, they may differ entirely in their factorial analysis.

LITERATURE CITED

BARTON, F. T., 1908 The cat: Its points, and management in health and disease. pp. xx + 106. London: Everett and Co.

BONHOTE, J. L., 1915 Vigour and heredity. pp. xii + 263. London: West, Newman and Co.

DONCASTER, L., 1904 On the inheritance of tortoiseshell and related colours in cats. Proc. Cambridge Phil. Soc. **13**: 35-38.

DONCASTER, L., 1913 On sex-limited inheritance in cats, and its bearing on the sex-limited transmission of certain human abnormalities. Jour. Genetics **3**: 11-23.

IBSEN, H. L., 1916 Tricolor inheritance. I. The tricolor series in guinea-pigs. Genetics **1**: 287-309.

JOHANNSEN, W., 1913 Elemente der exakten Erblichkeitslehre. 2. Aufl., pp. xi + 724. Jena: Gustav Fischer.

LITTLE, C. C., 1912 Preliminary note on the occurrence of a sex-limited character in cats. Science N.S. **35**: 784-785.

WHITING, P. W., 1915 The tortoiseshell cat. Amer. Nat. **49**: 518-520.

ON THE EFFECT OF SELECTION IN PARAMECIUM[1]

JAMES E. ACKERT

Kansas State Agricultural College, Manhattan, Kansas

[Received June 8, 1916]

In 1908, JENNINGS (p. 511) reported on the effect of selection in Paramecium as follows:

"Thus we come uniformly to the result in all our experiments that selection has no effect within a pure line (progeny of a single individual); the size is determined by the line to which the animals belong, and individual variations among the parents have no effect on the progeny."

While these results seemed conclusive, the fact that they were based upon separate lots of from one hundred to as few as a dozen individuals, together with the desirability of testing the principle independently, led to the present series of experiments in which somewhat larger numbers of Paramecia have been measured.

These experiments were begun in 1911 at the suggestion of Professor CHARLES ZELENY to whom I take pleasure in expressing my indebtedness for help and kindly criticism.

MATERIAL AND METHODS

Two species of Paramecia were used: *Paramecium caudatum,* the common large form having a single micro-nucleus and a pointed posterior end, and *P. aurelia,* the small species with two micro-nuclei and a blunt caudal end.

As the number of factors which may affect the metabolic processes, and incidentally the size of the animals, is large, the methods are given in some detail. In a typical experiment a Paramecium was placed in a few drops of hay infusion on a depression slide kept in a moist chamber. After several generations, the longest and the shortest Paramecium were selected from its descendants. This was accomplished by removing several of the largest and several of the smallest animals, making the final selections from these individuals. The Paramecia selected were isolated in equal quantities of infusion and kept under environmental

[1] Contribution from the Zoological Laboratory of the University of Illinois, No. 64, and from the Zoological Laboratory, Kansas State Agricultural College, No. 8.

conditions as nearly identical as it was possible to make them. As the descendants of each group increased in number, they were transferred to watch glasses. Later, all animals in each group were killed and measured. Separate capillary pipettes were used for transference, feeding, and killing. To lessen the harmful effects of excretion products, the Paramecia were transferred to fresh culture media every second day. In all cases, the transference pipette was rinsed in boiling water immediately after caring for each group to avoid the possibility of mixing cultures. The food consisted of separate infusions of timothy hay, alfalfa, wheat straw, oat straw, beef, and liver, left open to the air for twenty-four hours after boiling. Scrupulous care was taken to give the same quantity of food to each group of Paramecia under comparison.

For killing and preserving the animals, two kinds of fluids were used: (1) Worcester's fluid (a saturated solution of mercuric chloride in 10 percent formol), and (2) 10 percent formol. Both fluids killed instantly and preserved the animals satisfactorily. However, specimens kept in Worcester's fluid some weeks became partially imbedded in mercuric chloride crystals. No harmful effects have been noted from the use of 10 percent formol. In killing, approximately fifty individuals were placed in a small receptacle and overwhelmed with the hot killing fluid. All Paramecia in the groups under comparison were, as nearly as possible, killed at the same time.

Measuring was accomplished by two methods. By the first, the images of the animals were thrown upon a screen with a projection microscope, giving a magnification of three hundred and twenty diameters. The length and breadth of the image of each individual were measured with a millimeter ruler. Here each unit of measurement was equal to three and one-eighth microns. By the second, measurements of the animals were made with a compound microscope and ocular micrometer with a combination of lenses such as to make one unit of measurement equal to seven and one-half microns. In each method, the use of a checkered glass slide and a mechanical stage made it possible to locate all of the Paramecia without duplicating measurements. The well known formulæ brought together by DAVENPORT (1899) have been employed in computing the constants. All computations were checked by the aid of a Monroe mechanical calculator.

In making the polygons for length of groups A, B, and C (figs. 1, 3, 5), four units of measurement were grouped together to make a single unit on the abscissa of the polygon. This was done in order to destroy any irregularities due to an unconscious prejudice on the part of the

measurer for certain numbers. At the bottoms of these polygons, the numbers are the measurements in millimeters of the images of the Paramecia. In the polygons for breadth of groups A, B, and C (figures 2, 4, 6), two units of measurement were grouped together to make one unit on the abscissa of the polygon.

In the polygons for length and also those for breadth of groups D_1 and D_2 (figures 7, 8), two units of measurement were grouped together to make a single unit on the abscissa. There seemed to be little evidence of error due to unconscious preference for certain numbers in measuring the breadths of these groups. This was probably due to the greater ease of taking this measurement, since it was possible to hold both of its limits on the scale, in the eye at once. The numbers at the bottoms of the polygons of D_1 and D_2 (figures 7, 8) are in microns. In all of the polygons, the numbers to the left indicate numbers of Paramecia.

EXPERIMENTS

The main question in these experiments is: Can diverse groups of Paramecia be obtained by selection within a single group, i.e., if, from the progeny of a single animal, the largest and the smallest individual be selected, will there develop two diverse groups, one of greater mean size than the other? To answer this question, four extensive tests have been made. The groups, in which the selections were made, were designated as A, B, C, and D, respectively.

Experiment 1. The original Paramecium in group A (table 1) was a descendant of a *P. aurelia* taken from a stream seven months previously. The former was isolated on a depression slide in three drops of hay infusion. When its descendants numbered 110, two individuals were selected from them,—one, the shortest Paramecium, whose approximate length and breadth were 90 and 36 microns, respectively, the other, the longest Paramecium, approximately 162 microns in length and about 45 microns in breadth. The progeny of the shortest individual became known as group A_1, that of the longest as group A_2. When the descendants in each of these two groups numbered several hundred, they were killed and measured. The 705 animals of group A_1 had a mean length of 124.996 microns with a probable error of $\pm$0.281 microns and the 724 individuals of group A_2 had a mean length of 124.346 microns with a probable error of $\pm$0.300 microns. The average breadth of group A_1 was 55.050 $\pm$0.138 microns, that of group A_2 was 54.290 $\pm$0.146 microns.

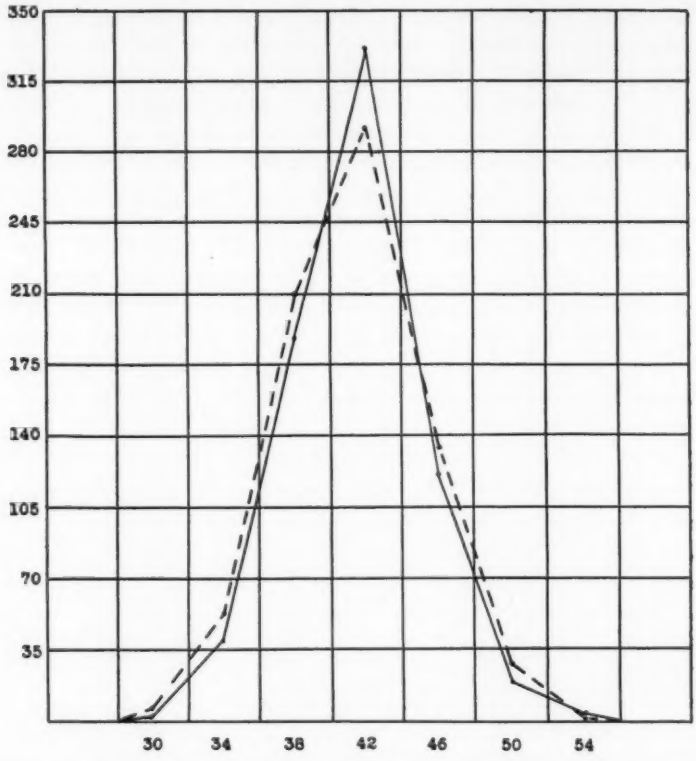


FIGURE 1.—Frequency polygons of the lengths of groups A_1 and A_2. Continuous line, A_1, number of Paramecia 705. Broken line, A_2, number of Paramecia 724.

From these data, it is seen that the longer ancestor gave individuals which were, on the average, slightly shorter than those derived from the shorter ancestor, but the difference is much less than three times the sum of the probable errors of the mean lengths and thus fails to come near the limit of what is ordinarily considered as significant. Similarly, the progeny of the larger ancestor have the smaller mean breadth. The difference appears to be without significance, since it is less than three times the sum of the probable errors of the means.

Experiment 2. The original *P. caudatum* in group B (table 2) was taken from a stream and isolated on a depression slide in a medium consisting of three drops of stream water, one drop of hay infusion,

TABLE 1.—*Diagram of experiment 1.*

Paramecium aurelia

A

I

110

Shortest Paramecium	Longest Paramecium
A_1	A_2
Approximate length, 90 microns	Approximate length, 162 microns
Approximate breadth, 36 microns	Approximate breadth, 45 microns
I	I
705	724
Killed and measured	Killed and measured
Length { Mean, 124.996 ± 0.281 microns; Stand. dev., 11.134 ± 0.196 microns	Length { Mean, 124.346 ± 0.300 microns; Stand. dev., 12.053 ± 0.213 microns
Breadth { Mean, 55.050 ± 0.138 microns; Stand. dev., 5.675 ± 0.087 microns	Breadth { Mean, 54.290 ± 0.146 microns; Stand. dev., 6.046 ± 0.106 microns

TABLE 2.—*Diagram of experiment 2.*

Paramecium caudatum

B

I

425

Shortest Paramecium	Longest Paramecium
B_1	B_2
Approximate length, 162 microns	Approximate length, 235 microns
Approximate breadth, 45 microns	Approximate breadth, 54 microns
I	I
703	923
Killed and measured	Killed and measured
Length { Mean, 179.540 ± 0.465 microns; Stand. dev., 18.325 ± 0.328 microns	Length { Mean, 174.762 ± 0.403 microns; Stand. dev., 18.275 ± 0.287 microns
Breadth { Mean, 56.359 ± 0.200 microns; Stand. dev., 7.878 ± 0.140 microns	Breadth { Mean, 57.640 + 0.168 microns; Stand. dev., 7.646 ± 0.115 microns

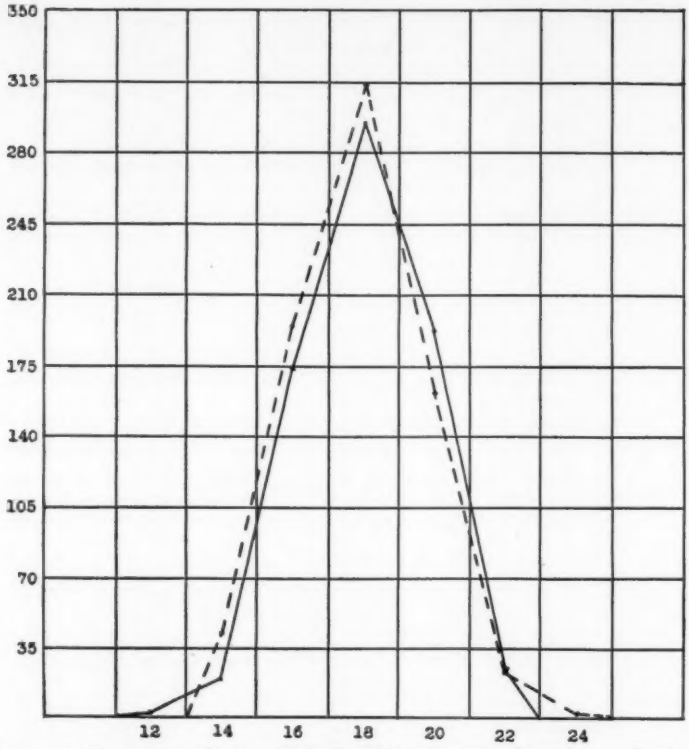


FIGURE 2.—Frequency polygons of the breadths of groups A_1 and A_2. Continuous line, A_1, number of Paramecia 705. Broken line, A_2, number of Paramecia 724.

and one drop of beef infusion. From approximately 425 of its descendants, one of the shortest Paramecia, whose approximate length and breadth were 162 and 45 microns, respectively, and one of the longest Paramecia, having a length of approximately 235 microns and a breadth of about 54 microns, were selected. The progeny of the shortest one were designated as group B_1, those of the longest as group B_2. When the descendants in each of these groups numbered several hundred, they were killed and measured. The 703 individuals of group B_1 had a mean length of 179.540 microns with a probable error of ± 0.465 microns and the 923 of group B_2 had a mean length of 174.762 microns with a probable error of ± 0.403 microns. The average breadth of group B_1 was 56.359 ± 0.200 microns, and that of B_2 was 57.640 ± 0.168 microns.

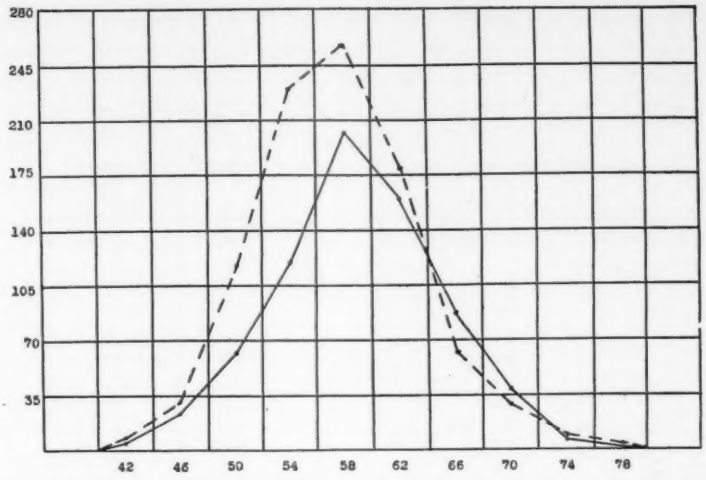


FIGURE 3.—Frequency polygons of the lengths of groups B_1 and B_2. Continuous line, B_1, number of Paramecia 703. Broken line, B_2, number of Paramecia 923.

An examination of these data shows that the longer ancestor gave descendants which were, on the average, slightly shorter than those derived from the shorter ancestor. But the fact that the progeny of the larger ancestor increased (approximately 3½ microns) in mean breadth may account, in part, for the slight difference in the lengths of these groups. At any rate, the descendants of the longest Paramecium selected have a smaller mean length than do the progeny of the shortest Paramecium selected. The measurements thus indicate a negative effect of selection, but obviously a more probable explanation is the existence of some uncontrolled environmental difference between the two cultures.

Experiment 3. The original *P. caudatum* in group C (table 3) was taken from a stream and isolated in a medium consisting of three drops of stream water, one drop of hay infusion and one drop of beef infusion. When its descendants numbered 380, selections were made as follows: (1) the shortest Paramecium with an approximate length and breadth of 162 and 36 microns, respectively, and (2) the longest individual with a length of approximately 198 microns and a breadth of about 45 microns. The progeny of the shortest animal was known as group C_1, those of the longest as group C_2. When the descendants in each of these two groups became numerous, they were killed and measured. The 815

TABLE 3.—*Diagram of experiment 3.*

Paramecium caudatum

C

I

380

Shortest Paramecium	Longest Paramecium
C_1	C_2
Approximate length, 162 microns	Approximate length, 198 microns
Approximate breadth, 36 microns	Approximate breadth, 45 microns
I	I
815	710
Killed and measured	Killed and measured
Length { Mean, 167.106 ± 0.465 microns; Stand. dev., 20.268 ± 0.031 microns	Length { Mean, 164.034 ± 0.496 microns; Stand. dev., 19.737 ± 0.353 microns
Breadth { Mean, 66.093 ± 0.184 microns; Stand. dev., 7.815 ± 0.122 microns	Breadth { Mean, 67.800 ± 0.231 microns; Stand. dev., 9.178 ± 0.162 microns

TABLE 4.—*Diagram of experiment 4.*

Paramecium aurelia

D

I

900

Shortest Paramecium	Longest Paramecium
D_1	D_2
Approximate length, 135 microns	Approximate length, 184 microns
Aproximate breadth, 60 microns	Approximate breadth, 64 microns
I	I
694	591
Killed and measured	Killed and measured
Length { Mean, 125.307 ± 0.354 microns; Stand. dev., 13.908 ± 0.250 microns	Length { Mean, 117.996 ± 0.355 microns; Stand. dev., 12.868 ± 0.250 microns
Breadth { Mean, 44.923 ± 0.159 microns; Stand. dev., 7.815 ± 0.122 microns	Breadth { Mean, 48.211 ± 0.348 microns; Stand. div., 8.482 ± 0.165 microns

individuals of group C_1 had a mean length of 167.105 microns with a probable error of ±0.465 microns and the 710 of group C_2 had a mean length of 164.034 microns with a probable error of ±0.496 microns. The average breadth of group C_1 was 66.093 ±0.184 microns, and that of C_2 was 67.800 ±0.231 microns.

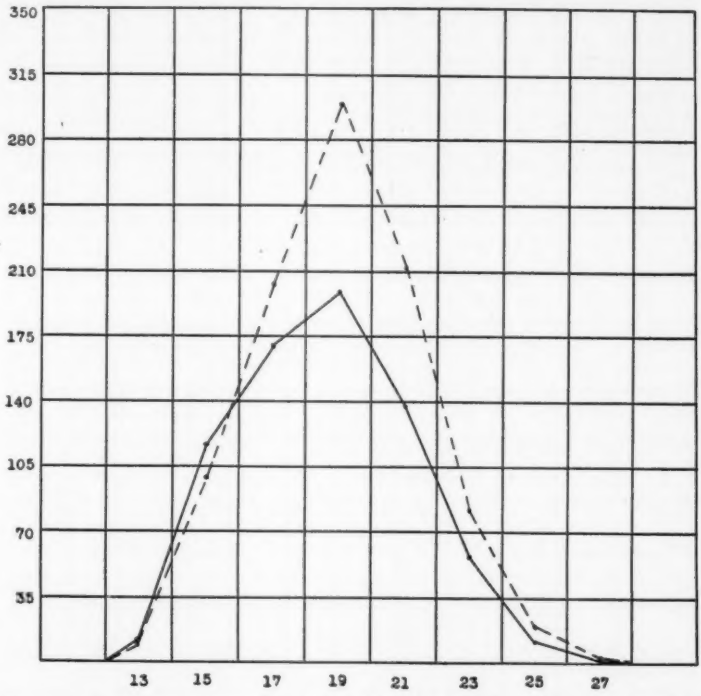


FIGURE 4.—Frequency polygons of the breadths of groups B_1 and B_2. Continuous line, B_1, number of Paramecia 703. Broken line, B_2, number of Paramecia 923.

These data show that the difference between the mean lengths of these two groups, is slightly more (0.088 microns) than three times the sum of the probable errors of their means, and thus exceeds the limit of what is ordinarily considered a significant difference. But, here, also, the longer ancestor gave descendants whose mean length was less than that of the progeny of the shorter ancestor, so that the difference can hardly be considered as significant. It seems probable that the difference may be due to an uncontrolled factor in the environment. A comparison of the

breadths shows a slight increase in favor of the descendants of the longer ancestor, but it seems doubtful if such a difference can be accepted as significant without question.

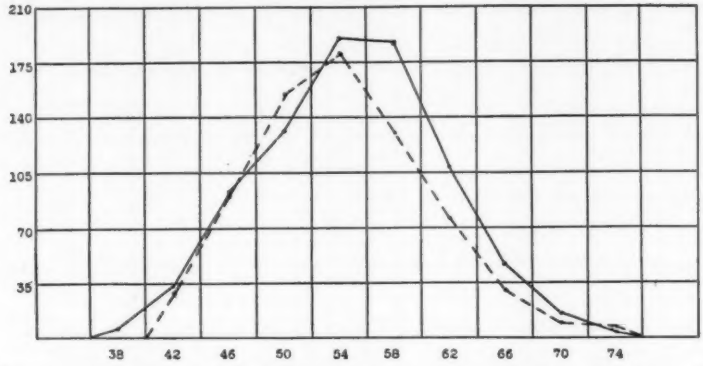


FIGURE 5.—Frequency polygons of the lengths of groups C_1 and C_2. Continuous line, C_1, number of Paramecia 815. Broken line, C_2, number of Paramecia 710.

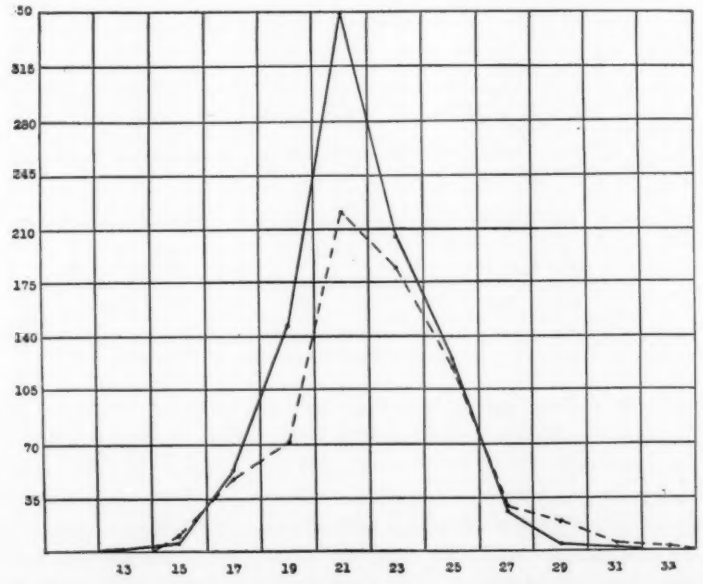


FIGURE 6.—Frequency polygons of the breadths of groups C_1 and C_2. Continuous line, C_1, number of Paramecia 815. Broken line, C_2, number of Paramecia 710.

Experiment 4. In making selections of shortest animals in previous experiments, it seemed possible that some of them might be young individuals, so a test was made with Paramecia that were known to be mature. The original *P. aurelia* (table 4, p. 394) of group D was taken from a laboratory culture and isolated in three drops of straw infusion. From approximately 900 of its descendants, five small Paramecia and five large ones were selected. Each of these ten individuals was placed in a separate receptacle and watched until the first sign of a constriction appeared, whereupon the approximate measurement of the animal was made. From these ten, two individuals were selected,—one, the shortest Paramecium whose approximate length and breadth were 135 and 60 microns, respectively, the other, the longest one, with a length of approximately 184 microns and a breadth of about 64 microns. The progeny of the shortest Paramecium was designated as group D_1, those of the longest as group D_2. When the descendants in each of these two groups numbered several hundred, they were killed and measured. The 694 individuals of group D_1 had a mean length of 125.307 microns with

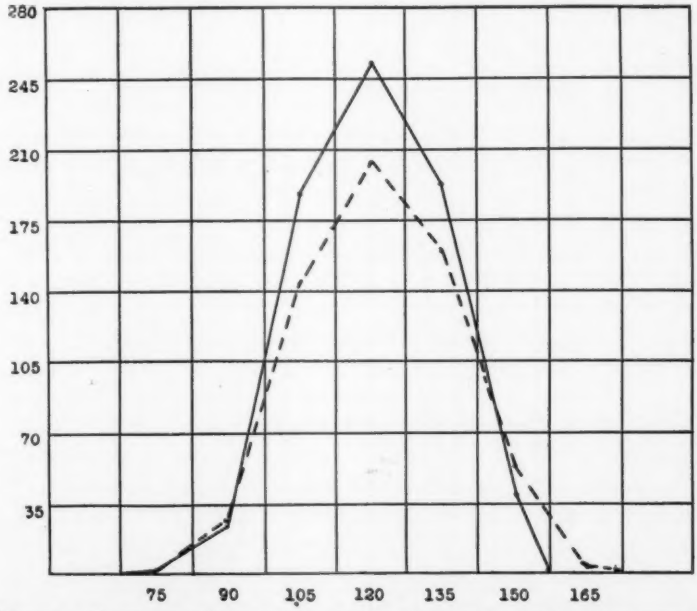


FIGURE 7.—Frequency polygons of the lengths of groups D_1 and D_2. Continuous line, D_1, number of Paramecia 694. Broken line, D_2, number of Paramecia 591.

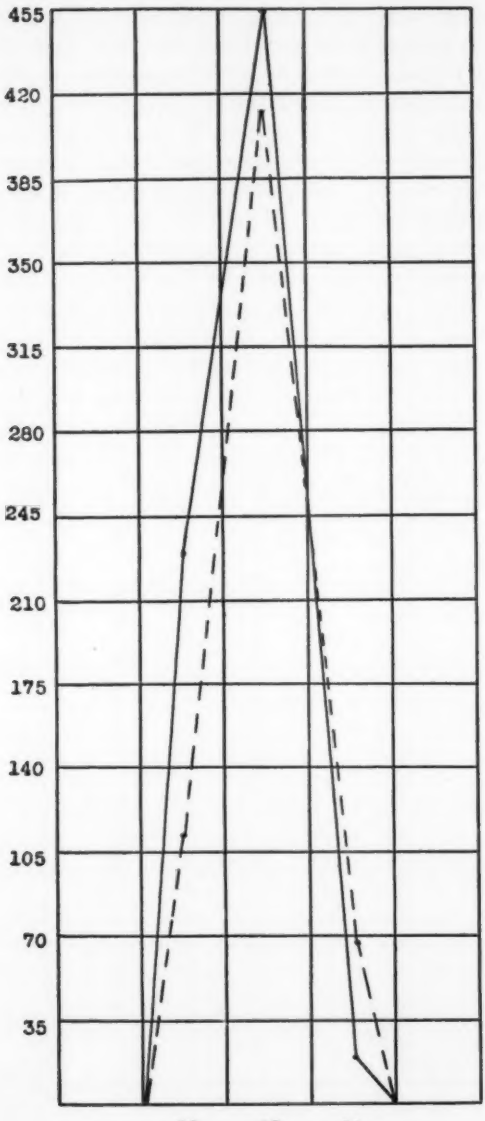


FIGURE 8.—Frequency polygons of the breadths of groups D_1 and D_2. Continuous line, D_1, number of Paramecia 694. Broken line, D_2, number of Paramecia 591.

a probable error of ±0.354 microns and the 591 of group D_2 had a mean length of 117.996 microns with a probable error of ±0.355 microns. The average breadth of D_1 was 44.923 ±0.159 microns, and that of D_2 was 48.211 ±0.348 microns.

An examination of these data shows that the longer ancestor gave descendants which were, on the average, slightly shorter than those derived from the shorter ancestor. But the fact that the mean breadth of group D_2 is greater than that of D_1, may account in part for the difference in the mean lengths of these groups. At any rate, the descendants of the longest Paramecium selected have a smaller mean length than do the progeny of the shortest Paramecium selected. The measurements thus indicate a negative effect of selection, but here, also, a more probable explanation is the existence of some uncontrolled environmental difference between the two cultures.

DISCUSSION AND CONCLUSIONS

From the results of these tests, it appears that diverse groups of Paramecia cannot be obtained by selection within the progeny of a single individual. Even when the difference in length between the shortest and the longest Paramecium selected is as much as 57 microns (average difference in the four tests), diverse groups failed to develop, each of the two groups under comparison either having the same mean lengths or the progeny of the longest Paramecium having the smaller mean length. When repeated differences in size such as these exist (i.e., when one of two animals under comparison is approximately twice as large as the other) with no corresponding difference appearing in the mean size of their respective progeny, and when similar results are obtained from large numbers of animals, it would seem obvious that variations in size in Paramecia descended from the same ancestor are not inherited, but that the mean size of the descendants is determined by the group to which the individuals belong.

In dealing with the question of how such variations arise, the effects of growth and of environment may be considered. No tests were made by the writer on variation of size due to growth, but JENNINGS (1908, table 15) has shown that the mean length of the progeny of a single Paramecium varies greatly at different ages. The difference in mean length of young individuals, two and one-half minutes after separation, and of adults of the same line eighteen hours old was more than 90 microns. These differences of size (due to growth) are almost sufficient

to explain the appearance of the variations without taking into account the effect of the environment. That the small Paramecia selected in the present experiments (groups A, B, and C) were young ones is obvious from the fact that the large animals selected divided first (table 5). At the end of the first three days, the descendants of the larger individuals were from one and one-half to two times as numerous as those of the smaller ones.

The environment presents a condition too complex to permit a full discussion here. It is known that the effect of the environment on the size of Paramecia may be considerable, especially when the animals are subjected to opposite extremes. JENNINGS (1908, table 18) shows that, by varying the amount of nutrition of animals in the same line, differences of 38 microns in mean lengths and 11 microns in mean breadths may be obtained. WOODRUFF (1911, p. 569) is of the opinion that "the variations in the daily rate in the different volumes of water is due to the excretion products of the Paramecia themselves." It is well known that the mean size of a group of individuals is affected by a change in division rate, for the more rapid the latter, the larger will be the number of young Paramecia present in the culture at that time, and vice versa. Even in cultures in which an attempt was made to keep environmental conditions constantly favorable, the effect on the mean size of the progeny of a single individual was noteworthy, as will be seen in the comparison which follows. A "new wild" *P. aurelia* (from which was descended group A of this series) was taken from a stream and isolated in straw infusion. Its descendants were kept under conditions similar to those in the tests. In two weeks, 799 (lot 21) of its progeny, taken at random, were killed and measured (table 6). The remainder of the culture was continued as before (except that alfalfa infusion was used instead of straw infusion). Two months later, 337 (lot 22) of them, also taken at random, were killed and measured (table 6). In five months, groups A_1 and A_2 (table 1), descendants of this line, were killed and measured. Even though these four lots were kept under favorable conditions, there was a difference in mean lengths of 25 microns (between lot 22 and group A_2 table 7,). The same two groups differed 19 microns in breadth.

The variations in size of Paramecia due to the combined effects of growth and environment are so great that the cause of the appearance of such variations cannot be in doubt. In the fourth experiment (table 4), it will be recalled that the selection was made from mature animals,

TABLE 5.—*Comparison of number of progeny of Paramecia during first three days after selection.*

Date	Group A		Group B		Group C		Group D	
	A_1 Smaller	A_2 Larger	B_1 Smaller	B_2 Larger	C_1 Smaller	C_2 Larger	D_1 Smaller	D_2 Larger
1st day isolated	1	1	1	1	1	1	1	1
2nd day	2	8	4	8	4	4	2	1
3rd day	14	28	12	18	10	20	6	4

TABLE 6.—*Comparison of different lots of the descendents of a single Paramecium (aurelia); all lots kept under favorable environmental conditions.*

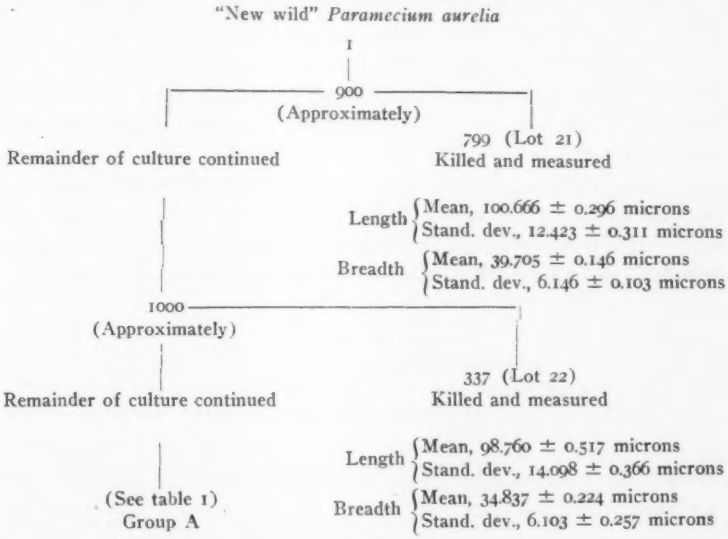


TABLE 7.—*Comparative lengths and breadths (in microns) of four groups of descendants of a "new wild" P. aurelia, all groups having been kept under favorable environmental conditions.*

	Lot 21	Lot 22	Group A_1	Group A_2
Mean length	100.666 ± 0.296	98.760 ± 0.517	124.996 ± 0.281	124.346 ± 0.300
Mean breadth	39.705 ± 0.146	34.837 ± 0.224	55.050 ± 0.138	54.296 ± 0.146

and that the result was negative. Here the factor of growth, of course, did not enter, but the differences in the respective lengths and breadths of the animals selected in group D were such as could readily be due to the environment. Hence, it seems evident that variations in size among descendants of a single Paramecium arise from the effects of growth and environment and that such variations are not inherited. It is possible that variations due to heredity may occur, but if they do, they are few in number compared with those arising from growth and environment, so that in the selections, variations due only to these two factors, are obtained.

A comparison of groups A and D (*P. aurelia*), and of B and C (*P. caudatum*) indicates that lines which differ in their characteristic mean sizes occur in nature (table 8).

In examining the results of the four experiments (table 8), it is seen that, in the first, the difference between the mean length of the progeny of the shortest Paramecium selected and of that of the longest one selected is less than three times the sum of the probable errors of their means and thus fails to reach the limit of what is ordinarily considered a significant difference. In each of the other three experiments (second, third, and fourth), the mean length of the progeny of the largest Paramecium was less than that of the descendants of the smallest one (table 8). Here, what was lost in length was compensated for, in part, by breadth. While the latter was not sufficient in any case to account for

TABLE 8.—*Mean sizes of the different groups of Paramecia under comparison.*

		Length in microns			Breadth in microns		
	Group	Parents	Offspring	Difference	Parents	Offspring	Difference
Group A, Experiment 1	A_1 A_2	90 162	124.996 ± 0.281 124.346 ± 0.300	0.650	36 45	55.050 ± 0.138 54.290 ± 0.146	0.760
Group B, Experiment 2	B_1 B_2	162 235	179.540 ± 0.465 174.762 ± 0.403	4.778	45 54	56.359 ± 0.200 57.640 ± 0.168	1.281
Group C, Experiment 3	C_1 C_2	162 198	167.105 ± 0.465 164.034 ± 0.562	3.071	36 45	66.093 ± 0.184 67.800 ± 0.231	1.707
Group D, Experiment 4	D_1 D_2	135 184	125.307 ± 0.354 117.996 ± 0.355	7.311	60 64	44.923 ± 0.159 48 211 ± 0.348	3.288

the difference in mean lengths, it is without doubt, significant. From these results and from the fact that the groups were large (minimum of 591, maximum of 923, total nearly 6000 individuals), it seems evident that selection within the progeny of a single Paramecium is without effect.

These results are in accord with those of certain other investigators working with organisms descended from single individuals. After making his well-known tests on self-fertilizing beans and barley, JOHANNSEN (1903) showed that, in nature, there are many pure lines, differing slightly from each other, but that selection within the progeny of a single seed had no effect upon the characteristics of that line. As stated elsewhere in this paper, JENNINGS (1908), in his extensive work on Paramecium, came to a similar conclusion in regard to the occurrence in nature of many "pure lines" differing slightly in their characteristic mean dimensions, and to the ineffectiveness of selection within a pure line. HANEL (1907), who experimented with *Hydra grisea* reproducing by budding, found strains differing with respect to numbers of tentacles. On selecting within a strain, however, such variations were not inherited. LASHLEY (1915) working with *Hydra viridis* found a slight difference, in the direction of selection, in the averages of the earlier buds of two groups, but this difference did not persist in the buds produced later by the same parents; regression was complete in a single generation. Even by continued selection in the same lines, LASHLEY (1916) was unable to obtain any positive results, selection within the progeny of a single Hydra being without effect. KELLY (1913) found in the parthenogenetic insect, *Aphis rumicis*, variations in the relative lengths of the third and fourth segments of the antennae. While his selections were too limited to be conclusive, they indicate that "the somatic differences in the parthenogenetic line are not inherited." EWING (1914) obtained similar results in parthenogenetic strains of *Aphis avenae*. In a given strain, he found large variations from the mean, but they were not transmitted to the offspring, selection within the descendants of a single parthenogenetic Aphis being without effect.

It is obvious that the findings of these investigators, together with the results of the present tests, support the conception of the non-variability of the character in inheritance. Clearly, these results do not support the recent statement of CASTLE (1916, p. 253) who says, "No race of animals has yet been shown to be devoid of genetic variations in size." So far as the writer has been able to ascertain, races of *Paramecium*

aurelia descended from single ancestors are not known to possess genetic variations in size, while JENNINGS (1908, table 23, i and its progeny), and the writer in this paper (experiments 1 and 4, table 8) have shown that variations in size among the progeny of a single *P. aurelia* are not inherited. However, evidence of genetic variations among the descendants of single organisms, reproducing asexually, has recently been set forth. CALKINS and GREGORY (1913) found heritable variations in size and in conjugating power within the progenies of single ex-conjugants of *Paramecium caudatum*. STOCKING (1915) has shown that, in the progeny of a single ex-conjugant of *Paramecium caudatum*, abnormalities were inherited through a large number of generations. In certain lines, however, such abnormalities were not hereditary. MIDDLETON (1915) by long continued selection within the progeny of a single *Stylonychia pustulata* (reproducing by fission), obtained two sets ('clones'?) differing characteristically from each other in rate of fission, under identical conditions. JENNINGS (1916), working on Difflugia, reproducing asexually, has found selection within the progeny of a single individual effective. From these findings, it is evident that certain morphological and physiological variations occurring within the progeny of a single individual (reproducing asexually) may be inherited. However, before drawing definite conclusions, tests should be made on still other kinds of uniparental organisms, using as bases of selection a large variety of characters.

SUMMARY

1. Variations in size of Paramecia descended from a single animal appear to be due to the environment and to growth.

2. In these experiments, in which nearly six thousand animals were measured, the results indicate that selection within the progeny of a single Paramecium is without effect. Even when one of the animals selected was twice as long as the other, diverse groups failed to develop, each of the groups under comparison either having the same mean lengths, or the progeny of the longest Paramecium having the smaller mean length.

LITERATURE CITED

CALKINS, G. N., and GREGORY, LOUISE H., 1913 Variations in the progeny of a single ex-conjugant of *Paramecium caudatum*. Jour. Exp. Zool. **15**: 467-525.

CASTLE, W. E., 1916 Size inheritance in guinea-pig crosses. Proc. Nat. Acad. Sci. **2**: 252-264.

DAVENPORT, C. B., 1899 Statistical methods, with special reference to biological variations. New York: John Wiley & Sons.

EWING, H. E., 1914 Pure line inheritance and parthenogensis. Biol. Bull. **26**: 25-35.

HANEL, E., 1907 Vererbung bei ungeschlechtlicher Fortpflanzung von *Hydra grisea*. Zeitschr. f. Naturw Jena **43**: 321-372.

JENNINGS, H. S., 1908 Heredity, variation and evolution in Protozoa II. Proc. Amer. Phil. Soc. **47**: 393-545.

1916 Heredity, variation and the results of selection in the uniparental reproduction of *Difflugia corona*. Genetics **1**:407—.

JOHANNSEN, W., 1903 Ueber Erblichkeit in Populationen und in reinen Linien v + 68 pp. Jena: Gustav Fischer.

KELLY, J. P., 1913 Heredity in a parthenogenetic insect (Aphis). Amer. Nat. **47**: 229-234.

LASHLEY, K. S., 1915 Inheritance in the asexual reproduction of Hydra. Jour. Exp. Zool. **19**: 157-210.

1916 Results of continued selection in Hydra. Jour. Exp. Zool. **20**: 19-26.

MIDDLETON, A. R., 1915 Heritable variations and the results of selection in the fission rate of *Stylonychia pustulata*. Jour. Exp. Zool. **19**: 451-504.

STOCKING, RUTH J., 1915 Variation and inheritance in abnormalities occurring after conjugation in *Paramecium caudatum*. Jour. Exp. Zool. **19**: 387-450.

WOODRUFF, L. L., 1911 The effect of excretion products of Paramecium on its rate of reproduction. Jour. Exp. Zool. **10**: 557-581.